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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12Q 1/68	A2	(11) International Publication Number: WO 99/25878
		(43) International Publication Date: 27 May 1999 (27.05.99)

(21) International Application Number: PCT/US98/24996

(22) International Filing Date: 19 November 1998 (19.11.98)

(30) Priority Data:
08/974,180 19 November 1997 (19.11.97) US

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: METHODS FOR MODULATING AND IDENTIFYING CELLULAR SENESCENCE

(57) Abstract

Human gene GC6 is expressed more abundantly in senescent cells than young cells. Isolated, purified, and recombinant nucleic acids and proteins corresponding to the human GC6 gene and its mRNA and protein products, as well as peptides and antibodies corresponding to the GC6 protein can be used to identify senescent cells, distinguish between senescent and young cells, identify agents that alter senescent gene expression generally and GC6 expression specifically; such agents as well as GC6 gene and gene products and products corresponding thereto can be used to prevent and treat diseases and conditions relating to cell senescence.

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METHODS FOR MODULATING AND IDENTIFYING CELLULAR SENESCENCE

BACKGROUND OF THE INVENTION

FIELD OF INVENTION

The present invention relates to cellular senescence, markers of cellular senescence, and methods and reagents for identifying senescent cells and agents that alter senescent gene expression of cells, as well as for detecting, diagnosing, preventing, and treating senescence-related diseases and conditions in humans and other mammals. The invention provides oligonucleotide probes and primers, polynucleotide plasmids or vectors, peptides, proteins, and antibodies relating to genes and gene products associated with the senescence in mammalian cells. The invention thus relates to the fields of molecular biology, chemistry, gerontology, pharmacology, oncology, and screening and diagnostic technology.

DESCRIPTION OF RELATED DISCLOSURES

Somatic cells have a finite replicative capacity (Hayflick and Moorhead, 1961, *Exp. Cell Res. 25*:585-621; Hayflick, 1965, *Exp. Cell Res. 37*:614-636; and Hayflick, 1970, *Exp. Geront. 5*: 291-303). This process is a major etiological factor in aging and age-related disease (Goldstein, 1990, *Science 249*:1129-1133; Stanulis-Praeger, 1987, *Mech. Aging Dev. 38*:1-48; and Walton, 1982, *Mech. Aging Dev. 19*:217-244). As cells undergo replicative senescence *in vitro* and *in vivo*, cells not only lose the ability to divide in response to growth stimuli, but also exhibit significant deleterious changes in the pattern of gene expression (West, 1994, *Arch. Derm. 130*:87-95). As an individual grows older, senescent cells make up an increasing percentage of the cells present in the tissues of the aging individual. The altered pattern of gene expression exhibited by senescent cells contributes significantly to age-related pathologies. Reversal of, or a delay in the onset of, senescence provides an effective therapy for diseases in which replicative senescence is a factor.

One fundamental cause of cellular senescence is the progressive loss of telomeric DNA in somatic cells that lack the enzyme, telomerase (Nakamura *et al.*, 15 Aug. 1997, *Science 277*:955 et seq.). This arrest appears to be mediated by a DNA checkpoint by which the cell recognizes the shortened telomere as damaged DNA and causes cell cycle arrest similar to that observed in normal cells after DNA damage.

As cells progress to a senescent state, the cells exhibit an elongation of the G1 phase of the cell cycle, leading to a longer cell time of cycle transit. As the progression from a mitotically active to a senescent state continues, cells fail to respond to mitotic signals and remain in G1. This inability of senescent cells to enter the cell cycle represents a significant difference between young and old cells. Unlike old cells, young cells become quiescent entering G0 but can be subsequently induced to reenter the cell cycle and divide. However, senescent cells, while remaining viable and metabolically active, become refractile to entering the cell cycle.

A characteristic of replicative senescence is that changes in the pattern of gene expression can be observed as the cell progresses through its replicative lifespan. These changes are reflected in a decrease in the expression of "young-specific" genes and an increase in the expression of "old-specific" genes. Together, these young- and old-specific genes are referred to herein as "senescence-specific" genes, where a senescence-specific gene is any gene for which the product

of the gene is differentially expressed between young quiescent cells and senescent cells. Not only do these changes affect the structure and function of the senescent cell, but also such changes can influence the physiology of surrounding cells and the tissue matrix by altering the extracellular environment, *i.e.*, in a paracrine fashion through the release of different proteins or through changes in cell-cell interactions. Several senescence-specific genes have been described (Linskens *et al.*, PCT No. WO 96/13610, published 9 May 96 and incorporated herein by reference).

Changes in mRNA levels and cellular content of specific proteins provides evidence of a senescence-specific program of gene expression, leading to a differentiated genotypic and phenotypic state (Linskens *et al.*, supra). Changes in steady state mRNA levels can translate into changes in protein expression and levels, as the rate and extent of mRNA translation represents an additional mechanism of controlling gene expression at the protein level. Cell structure and function also change when gene expression at the protein level changes.

Thus, as an individual grows older and the percentage of senescent cells in the aging individual's tissues increases, the resultant altered pattern of gene expression can contribute significantly to the pathophysiology of age-related changes in specific tissues (*e.g.*, skin) and age related diseases. There is a need for therapeutic agents and treatments targetting the underlying biology of aging and age-related diseases, particularly the biology relating to the fundamental changes in gene expression that occur with cell senescence and lead to the development of age-related disease. The present invention helps meet that need by providing new methods and agents for discriminating between young and senescent cells, for identifying agents that modulate senescent gene expression, and for treating diseases induced or exacerbated by cellular senescence.

SUMMARY OF THE INVENTION

The present invention provides methods and reagents for identifying compounds and compositions that alter senescent gene expression and for identifying or distinguishing senescent cells from non-senescent cells, as well as methods, reagents, and compositions that are specific and effective for treating conditions and diseases associated with cellular senescence. In particular, the invention provides methods, reagents, and compounds relating to a novel senescence-related gene referred to herein as GC6. The methods, reagents, and compositions of the invention can be applied to a wide variety of cell types.

The invention provides synthetic and recombinant oligonucleotides and nucleic acids in a variety of forms, *i.e.*, isolatable, isolated, purified, or substantially pure, and for a variety of purposes, *i.e.*, as probes, primers, polynucleotides, and plasmid or vector nucleic acids. Thus, the invention provides recombinant or synthetic nucleic acids comprising at least about 8 to 10 to 15 to 25 to 100 or more contiguous nucleotides substantially identical or complementary in sequence to a contiguous nucleotide sequence of the human GC6 gene.

Nucleic acids and polynucleotides comprising such sequences can be isolated from plasmid pCIneoGC6 (available from the American Type Culture Collection, ATCC, under accession number ATCC 209608); an ~3 kb XhoI-XbaI restriction fragment of this plasmid comprises the complete coding sequence of the GC6 gene, which encodes a protein, designated pGC6, present in human cells. Homologues of the pGC6 protein and the GC6 gene are present in other mammalian cells and are also provided by the invention.

The recombinant expression vectors of the invention typically comprise at least about 25 to 100 to 1000 or more, up to the full length coding or open reading frame sequence, contiguous nucleotides identical or complementary in sequence to a contiguous nucleotide sequence of the

human GC6 gene. In other embodiments, the invention provides recombinant expression vectors that encode the promoter of the GC6 gene operably linked to the coding sequence of pGC6 or to a coding sequence for a reporter molecule, such as beta-galactosidase or secreted alkaline phosphatase, or other coding sequence not naturally associated with the GC6 gene promoter. The GC6 gene promoter can be isolated from libraries of human genomic DNA by probing with probes of the invention and identifying clones with inserts including all or a portion of the ORF of the gene and 5' upstream sequences or with primers of the invention using techniques such as 5'-RACE.

The expression vectors have many useful applications, including for production of the protein, pGC6, peptides derived from the protein, oligonucleotides and nucleic acids identical or complementary to at least a portion of the pGC6 coding sequence or other regions of the GC6 gene, or heterologous polynucleotides or polypeptides. The expression vectors of the invention can also be used in therapeutic methods of the invention for altering senescent gene expression, either to upregulate, down-regulate, or inhibit expression of pGC6 or mRNA transcribed from the GC6 gene in a target cell or tissue. Such expression vectors also include those that encode variants or "muteins" of pGC6 proteins, *i.e.*, express proteins that differ from pGC6 by deletion, substitution, and/or addition of one or more amino acids, as well as those that encode useful nucleic acids, such as antisense, ribozyme, and triple-helix forming nucleic acids that target GC6 gene products, *e.g.*, mRNA or pGC6. The recombinant expression vectors of the invention can be employed in recombinant host cells, cell free transcription and/or translation system, and in intact mammals, including humans.

The recombinant host cells and vectors of the invention are also useful in screens to identify agents that prevent or modulate senescent gene expression. For example, the vectors can be used to produce recombinant pGC6 that is employed in assays to identify agents that inhibit its function in cells. Also, vectors that employ the promoter of the GC6 gene to drive expression of a reporter molecule are useful in screens to identify agents that modulate the activity of the promoter of the GC6 gene.

As indicated above, the present invention also provides peptides and proteins corresponding to all or part of pGC6. While such compounds can be produced by recombinant methods using the vectors, host cells, and translation systems of the invention, the peptide and protein compounds of the invention, similarly to the oligonucleotides and vectors of the invention, can also be produced by synthetic means. In particular, the invention provides synthetic and recombinant peptides and proteins comprising at least about 6 to 10 to 15 to 25 to 100 or more, up to the full length of pGC6, contiguous amino acids identical or substantially identical in sequence to an amino acid sequence encoded by the GC6 gene. The present invention also provides methods for isolating the proteins and peptides of the invention in isolated or purified form from host cells and translation systems expressing recombinant pGC6.

The proteins and peptides of the invention can be used to generate antibodies specific for pGC6 and for particular epitopes specific to this protein. Thus, the invention provides polyclonal and monoclonal antibodies that specifically bind to pGC6. These antibodies can in turn be used to isolate pGC6 from normal or recombinant cells and so are useful in methods of the invention to purify the protein as well as other proteins associated therewith. These antibodies also have important application in the detection of cells comprising pGC6 in complex mixtures of cells. Such detection methods have application in screening, diagnosing, and monitoring diseases and other conditions associated with aging, such as hypertension, particularly as pGC6 exhibits significant homology to dopamine beta-hydroxylase (DBH). See Berkowitz *et al.*, 1988, *J. Pharm. Exp. Ther. 245*(3):850-7; Frigon and Stone, 10 Oct. 1978, *J. Biol. Chem. 253*(19):6780-6786;

Ohlstein *et al.*, 1987, *J. Pharm. Exp. Ther. 241*(2):554-9; Robertson *et al.*, July 1991, *Hypertension 18*(1):1-8; and Li *et al.*, 1995, *Biochem J.: 313*:57-64.

The present invention provides methods for identifying agents, *i.e.*, test compounds and compositions, that can prevent or alter the pattern of senescent gene expression in mammalian, especially human cells, which method comprises: (a) contacting said cells with an agent; (b) measuring an amount of a GC6 gene product in said treated cells to obtain a measured amount, *i.e.*, by using a GC6 gene product-specific nucleic acid primer or probe or antibody or assay of the invention; (c) comparing said measured amount with a control amount of said GC6 gene product determined by measuring said GC6 gene product in said cells in absence of said agent; and (d) identifying as an agent that can alter said pattern of senescent gene expression in cells as those agents that produce an increased or decreased measured amount relative to said control amount.

Any of a variety of GC6 gene products can be used in the method, for example, one can measure an amount of GC6-specific mRNA or pGC6 or one can measure an activity of pGC6. In addition, the assay or screen can be conducted in combination with analysis of the affect of the agent on other known senescent specific markers, such as, for example, beta-galactosidase, collagenase, interferon gamma, collagen I, collagen III, elastase, elastin, TIMP3, or IL-Ia, autofluorescence, acridine-orange fluorescence, and telomere length. A preferred method involves the measurement of a combination of markers including a GC6 gene product.

The present invention also provides methods for identifying or distinguishing senescent cells from young (also referred to as quiescent, presenescent, or non-senescent) cells in tissues or in culture. In general, a young cell produces lower amounts of GC6 gene products than a senescent cell. Thus, the present invention allows one to identify young cells, to identify senescent cells, to determine where in the development from young to fully senescent a cell or tissue sample is located, to identify the proportion or relative amounts of young and senescent cells in a sample, and to distinguish young from senescent cells. One such method comprises contacting a GC6 gene product within a cell or tissue with an agent that binds specifically to said GC6 gene product under conditions such that said agent and said GC6 gene product bind to one another; determining whether specific binding has occurred; and correlating the presence of senescent and non-senescent cells with the occurrence of binding.

The methods of the invention are broadly applicable to the identification of senescent cells and alteration of senescent gene expression in cells and tissues of mammals. The methods are especially useful and applicable to the identification of senescent cells and alteration of senescent gene expression in samples of biological material obtained from humans. Such samples will contain cells or cellular materials and will typically be obtained from humans for the purposes of determining agents that alter senescence gene expression and diagnosing or treating diseases or disease conditions induced or exacerbated by cell senescence. These and other aspects of the invention are described in more detail below.

DETAILED DESCRIPTION OF THE INVENTION

To facilitate understanding of the invention, the disclosure of the invention is organized in sections as follows. First, a glossary is provided to define terms and phrases used throughout the specification and claims. The next section describes useful reagents and compounds of the invention, which include oligonucleotides, recombinant expression vectors, peptides and proteins, as well as methods employing the same. The next section describes methods of the invention for identifying compounds that alter senescent gene expression; such compounds are referred to as modulators. The following section describes methods for identifying, or distinguishing between,

senescent and non-senescent cells, followed by a description of the diagnostic and therapeutic methods and applications of the invention, as well as compounds and compositions effective for treating conditions and diseases associated with senescent gene expression and aging by modulating expression levels of senescent specific genes. The specification concludes with a number of examples relevant to the invention and its practice and application.

I. GLOSSARY

Unless otherwise specified, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the invention pertains. Commonly used terms herein for purposes of describing how to make and use present invention are defined below.

"Affinity purification" refers to the purification or molecular separation of a compound from one or more, typically many more, compounds and compositions with which the compound desired to be purified is associated by binding to an agent that specifically binds the desired compound relative to the other compounds and compositions in the unpurified mixture. Typically, such binding is reversible, and the binding agent or compound is a biomolecule, *i.e.*, an oligonucleotide or antibody. For example, one type of binding agent is an "affinity oligonucleotide" (*i.e.*, an antisense oligonucleotide is an affinity oligonucleotide related to nucleic acids that comprise a complementary sequence of nucleotides). After binding of the affinity purification reagent to the compound to be purified, one typically, but not always, displaces the compound from the binding agent in a step referred to as elution. For example, one can use a "displacement oligonucleotide" to elute a desired nucleic acid from its specific binding agent.

An "agent" or "compound" refers to a chemical compound or composition, including, but not limited to, organic molecules, polynucleotides, proteins, peptides, and the like, a mixture of chemical compounds, an array of spatially localized compounds (*e.g.*, a peptide array, polynucleotide array, and/or other molecular array; where "array" refers to a collection of different molecular species, which can be immobilized on a surface), a biological macromolecule, a bacteriophage peptide display library, a bacteriophage antibody (*e.g.*, scFv) display library, a polysome peptide display library, or an extract made from biological materials such as bacteria, plants, fungi, or animal (particularly mammalian) cells or tissues.

An "antibody" refers to a naturally occurring or recombinant polypeptide or associated polypeptides and proteins that specifically bind to or "recognize" an analyte or "antigen". Naturally occurring antibodies are encoded by immunoglobulin genes, but a wide variety of antibodies and antibody-like molecules and fragments thereof, are available through recombinant DNA technology. Immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as myriad immunoglobulin variable region genes. An antibody can exist as an intact immunoglobulin or as any one of a number of well characterized fragments, *e.g.*, Fab' and F(ab)'2 fragments, and such antibodies can be produced by various means, including, as noted above, recombinant DNA methodology and from naturally occurring antibodies; for example, antibody fragments can be produced by digestion with various peptidases. See also, Harlow and Lane Antibodies, A Laboratory Manual, Cold Spring Harbor Publications, New York (1988), incorporated herein by reference.

An "antigenic determinant" or "epitope" refers to a particular chemical group or groups of an antigen that binds to a particular antibody and confers antigenic specificity. When a protein or fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies that bind specifically to a given region or three-dimensional

structure on the peptide or protein; these regions or structures are referred to as antigenic determinants. An antigenic determinant may compete with the intact antigen (*i.e.*, the immunogen used to elicit the immune response) for binding to an antibody.

"Antisense" refers to oligonuleotides or polynucleotides comprising sequences of nucleotides that are complementary to a sequence in another oligonucleotide or polynucleotide (*e.g.*, mRNA). Antisense oligonucleotides can be produced by a variety of methods, as is commonly known in the art. For example, but not limitation, antisense RNA can be synthesized by splicing the gene(s) or coding sequence of a gene of interest in a reverse orientation, relative to its orientation in nature, to a promoter that directs the synthesis of the antisense nucleic acid. An antisense oligonucleotide can bind to a complementary sequence in its "target" nucleic acid, such as a naturally occurring mRNA produced by a cell, via hydrogen bonding to form a duplex or double-stranded nucleic acid. Such duplex formation can reduce or completely inhibit the translation of proteins from the target mRNA or, if the antisense oligonucleotide is bound to DNA in a gene, transcription of that gene. In this manner, alteration or modulation of gene expression can be achieved.

"Biologically active" refers to compounds, such as, for example but without limitation, organic molecules such as drugs and synthetic or recombinant nucleic acids, peptides, proteins or the like, that exhibit an activity, *i.e.*, inhibit or increase transcription or translation of a nucleic acid or activity of an enzyme or property of a structural molecule, in a cell extract, cell, tissue, organism or animal.

A "cDNA" refers to a deoxyribonucleic acid produced by reverse-transcription and for double-stranded cDNA second-strand synthesis of mRNA or other RNA produced by a gene, as well as recombinant or synthetic replicas or derivatives thereof.

"Complementary" or "complementarity" refers to the well known property of nucleic acids arising out of hydrogen bonding or "base-pairing" between nucleotides to form double-stranded complexes. A first oligonucleotide or polynucleotide is complementary to or has complementarity with a second oligonucleotide or polynucleotide if the sequence of nucleotides in the first and second molecules allows them to form a stable duplex arising out of hydrogen bonding between A (adenosine) residues in one with T (thymidine) residues in the other and C (cytosine) residues in one with G (guanosine) residues in the other. By way of illustration only (because typically longer sequences of complementarity are required) the oligonucleotide having sequence "5'-A-G-T" is complementary to an oligonucleotide having the sequence "5'-T-C-A." Complementarity may be "partial," in which only some of the nucleic acid bases in an oligonucleotide are hydrogen bonded to bases in the other oligonucleotide, or "complete," in which all of the nucleic acids in one of the oligonucleotides are hydrogen bonded to a contiguous sequence of nucleotides in the other. The formation of a duplex nucleic acid from two single-stranded nucleic acids (such as an oligonucleotide probe to a target mRNA) is often referred to as "hybridization." Hybridization can be carried out or prevented by a variety of conditions, the cumulative effect of which is often referred to as "the stringency" of hybridization. Factors and conditions such as the length and composition (DNA, RNA, base composition) of the probe and target, the concentration of salts and other components (*e.g.*, the presence or absence of formamide, dextran sulfate, polyethylene glycol), temperature, solvent, and time of hybridization all contribute to the stringency of hybridization, as is well known in the art. Conditions of low stringency allow for hybridization of nucleic acids of partial complementarity, while conditions of high stringency can select for complete hybridization of a probe to a target nucleic acid. Thus, "stringent conditions" or "stringency" can refer to temperature and ionic conditions used in nucleic acid hybridization. Generally, stringent conditions are selected to be about 5 to 20 degrees C lower than the thermal

melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength and pH) at which half of a population of complementary double-stranded nucleic acid molecules becomes dissociated into single stranded molecules. The equation for calculating the Tm of nucleic acids is well known in the art. See, *e.g.*, Anderson and Young, Quantitative Filter Hybridization, in Nucleic Acid Hybridization (1985).

"Encoding" refers to an inherent property of a sequence of nucleotides in a nucleic acid, such as a gene in a chromosome or an mRNA, to serve as a template for synthesis of other polymers and macromolecules in biological processes having a defined sequence of nucleotides (rRNA, tRNA, other RNA molecules) or amino acids (peptides and proteins). For example, a gene encodes a protein if transcription and translation of mRNA produced by that gene produces the protein in a cell or other biological system. Both the coding strand of the gene, the strand having the nucleotide sequence identical to the sequence of nucleotides in the mRNA produced by the gene, and the complementary, non-coding strand of the gene can be referred to as encoding the protein or other product of that gene. Due to the degeneracy of the genetic code, a variety of different nucleic acid sequences can encode the same peptide or protein. Also, genes and mRNA of mammals (and other organisms) can include non-coding sequences called introns but still be referred to herein as "encoding" a peptide or protein.

An "expression control sequence" refers to a nucleotide sequence in a nucleic acid that regulates the expression (transcription and/or translation) of a nucleotide sequence operatively linked thereto. Expression control sequences can include, for example and without limitation, sequences of a promoter, enhancer, and transcription terminator, all of which can be involved in transcription of DNA to form RNA, and a ribosome-binding site, start codon (*i.e.*, ATG), splicing signal for an intron/exon, and a stop codon, all of which can be involved in translation of RNA to form a protein.

An "immunoassay" refers to an assay in which an antibody or fragment thereof is used to detect an analyte.

"Immunologically active" refers to the ability of natural, recombinant, or synthetic protein or peptide (or a nucleic acid) to induce an immune response in an animal.

A "label" refers to a compound or composition that facilitates detection of a compound or composition with which it is specifically associated, which can include conferring a property that makes the labeled compound or composition able to bind specifically to another molecule. "Labeled" refers to a compound or composition that is specifically associated, typically by covalent bonding but non-covalent interactions can also be employed to label a compound or composition, with a label. Thus, a label may be detectable directly, *i.e.*, the label can be a radioisotope (*e.g.*, ^{3}H, ^{14}C, ^{32}P, ^{35}S, ^{125}I, ^{131}I) or a fluorescent or phosphorescent molecule (*e.g.*, FITC, rhodamine, lanthanide phosphors), or indirectly, *i.e.*, by enzymatic activity (*e.g.*, horseradish peroxidase, beta-galactosidase, luciferase, alkaline phosphatase) or by its ability to bind to another molecule (*e.g.*, streptavidin, biotin, an antigen, epitope, or antibody). Incorporation of a label can be achieved by a variety of means, *i.e.*, by use of radiolabeled or biotinylated nucleotides in polymerase-mediated primer extension reactions, epitope-tagging via recombinant expression or synthetic means, or binding to an antibody. Labels can be attached directly or via spacer arms of various lengths, *i.e.*, to reduce steric hindrance. Any of a wide variety of labeled reagents can be used for purposes of the present invention. For instance, one can use one or more labeled nucleoside triphosphates, primers, linkers, or probes. The term label can also refer to a "tag", which can bind specifically to a labeled molecule. For instance, one can use biotin as a tag and then

use avidinylated or streptavidinylated horseradish peroxidase (HRP) to bind to the tag, and then use a chromogenic substrate (*e.g.*, tetramethylbenzamine) to detect the presence of HRP. In a similar fashion, the tag can be an epitope or antigen (*e.g.*, digoxigenin), and an enzymatically, fluorescently, or radioactively labeled antibody can be used to bind to the tag.

"Naturally occurring" refers to a substance, typically an amino acid, nucleotide, nucleic acid, or protein that exists in nature without human intervention. For example, deoxyribonucleic acid or DNA is naturally occurring. If a naturally occurring substance is produced by human intervention, the resulting substance is referred to as "synthetic" or "recombinant."

An "oligonucleotide" refers to a polymer of composed of nucleotides. Naturally occurring oligonucleotides include DNA and RNA and, as discussed above, can exist as single-stranded polymers or as duplexes, triplexes, or higher-order compositions of single-stranded polymers. An oligonucleotide can also be referred to as a polynucleotide or nucleic acid; typically, the latter terms are used to describe oligonucleotides of substantial (*i.e.*, 50 to 100 to 1000 to 10,000 or more nucleotides in) length, while the term oligonucleotide is most often used to refer to a polynucleotide from about 8 to 30 nucleotides in length. Oligonucleotide, as used herein, also refers to synthetic and non-naturally occurring forms or analogues of naturally occurring oligonucleotides, and such analogues can differ, relative to their naturally occurring counterparts, either in the linkages between nucleotides or in the base or sugar components of the nucleotides. For example, but without limitation, synthetic oligonucleotides include peptide-nucleic acids (PNAs), phosphoramidates, phosphorothioates, methyl phosphonates, 2-O-methyl ribonucleic acids, and the like. See also, Nielsen *et al.*, 1993, *Anticancer Drug Des. 8*:53-63, incorporated herein by reference. Polynucleotides and fragments or analogs thereof, may be prepared according to methods known in the art and described in Maniatis *et al.*, Molecular Cloning: A Laboratory Manual, 2nd Ed., (1989), Cold Spring Harbor, N.Y., and Berger and Kimmel, Methods in Enzymology, Volume 152, Guide to Molecular Cloning Techniques (1987), Academic Press, Inc., San Diego, CA, which are incorporated herein by reference.

The phrase "open reading frame" or "coding sequence" refers to a nucleotide sequence that encodes a polypeptide or protein. Such sequences are typically bordered on the 5'-end by an initiation codon (ATG) or another codon that does not encode a stop codon and on the 3'-end by a stop codon.

A "pharmaceutical composition" refers to a composition suitable for pharmaceutical or therapeutic use in a mammal. A pharmaceutical composition comprises a pharmacologically effective amount of an active agent and typically a pharmaceutically acceptable carrier.

The phrase "pharmaceutically acceptable carrier" refers to a pharmaceutical carrier, buffer, or excipient, such as a phosphate buffered saline solution, 5% aqueous solution of dextrose, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents and/or adjuvants. Suitable pharmaceutical carriers and formulations are described in Remington's Pharmaceutical Sciences, 19th Ed. (Mack Publishing Co., Easton, 1995). Preferred pharmaceutical carriers depend upon the intended mode of administration of the active agent. Typical modes of administration include enteral, oral, parenteral, subcutaneous, intramuscular, intravenous, intraperitoneal, topical, transdermal, or transmucosal administration.

"Pharmacologically effective amount" refers to that amount of an agent effective to produce the intended pharmacological result.

"Physiological conditions" refer to temperature, pH, ionic strength, viscosity, and like biochemical parameters compatible with a viable organism, such as those that exist intracellularly in a viable mammalian cell. For example, the intracellular conditions in a mammalian cell grown under typical laboratory culture conditions are physiological conditions. Suitable *in vitro* reaction conditions for PCR and many polynucleotide enzymatic reactions and manipulations are generally physiological conditions. In general, *in vitro* physiological conditions are 50-200 mM NaCl or KCl, pH 6.5-8.5, 20-45 degrees C, and 0.001-10 mM divalent cation (*e.g.*, Mg++, Ca++); preferably about 150 mM NaCl or KCl, pH 7.2-7.6, 5 mM divalent cation, and often include 0.01-1.0 percent protein (*e.g.*, BSA). A non-ionic detergent (Tween, NP-40, Triton X-100) can also be present, usually at about 0.001 to 2%, typically 0.05-0.2% (v/v). Particular aqueous conditions may be selected by the practitioner according to conventional methods. For general guidance, the following buffered aqueous conditions may be applicable: 10-250 mM NaCl, 5-50 mM Tris HCl, pH 5-8, with optional addition of divalent cation(s) and/or metal chelators and/or nonionic detergents and/or membrane fractions and/or antifoam agents and/or scintillants.

"Polymerase" refers to any enzyme capable of catalyzing a polymerization reaction, as of, for example, nucleotide to polynucleotides. It is intended that the term encompass any polymerase suitable for use in the amplification of nucleic acids of interest, including DNA polymerases such as *Taq* DNA polymerase obtained from *Thermus aquaticus*, although other polymerases, both thermostable and thermolabile, are also encompassed by this definition.

"Polypeptide" or "peptide" or "protein" refers to a polymer of amino acid residues and to variants and synthetic analogs of the same and are used interchangeably herein. Thus, these terms apply to amino acid polymers in which one or more amino acid residues is a synthetic non-naturally occurring amino acid, such as a chemical analog of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers.

"Primer" refers to an oligonucleotide, *i.e.*, a purified restriction fragment or a synthetic oligonucleotide, capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a primer extension product complementary to a nucleic acid strand (the "template") is induced, *i.e.*, in the presence of nucleotides and an agent for polymerization, such as DNA polymerase, and at a suitable temperature and pH. The primer is preferably single-stranded for maximum efficiency in amplification but may alternatively be double-stranded. If double stranded, the primer may need to be treated to separate its strands before being used to prepare extension products. Primers are typically oligodeoxyribonucleotides, but a wide variety of synthetic and non-naturally occurring oligonucleotide primers can be used for various applications. The preferred length of a primer depends on many factors, including application, temperature to be employed, template, reaction conditions, other reagents, and source. For example, depending on the complexity of the target sequence, an oligonucleotide primer typically contains 15-25 or more nucleotides, although it may contain fewer or more nucleotides. Short primer molecules generally require cooler temperatures to form stable hybrid complexes with template. Primers can be large polynucleotides, such as from about 200 nucleotides to several kilobases or more in length. A primer must be sufficiently complementary to the sequence on the template to which it is to hybridize to serve as a site for the initiation of synthesis but need not reflect the exact sequence of the template. For example, non-complementary nucleotides may be attached to the 5'-end of the primer, with the remainder of the primer sequence being complementary to the template. Alternatively, non-complementary nucleotides or longer sequences can be interspersed into a primer, provided that the primer sequence has sufficient complementarity with the sequence of the template to hybridize therewith and thereby form a template for synthesis of the extension product of the primer.

"Probe" refers to a molecule that binds to a specific sequence or sub-sequence of monomers in a polymer or to a moiety of another molecule. A probe can be, for example, an oligonucleotide that is capable of hybridizing to another oligonucleotide or polynucleotide of interest, often called the "target", through complementary base pairing, and such a probe can bind target nucleic acids lacking complete sequence complementarity with the probe, depending upon the stringency of the hybridization conditions. Oligonucleotide probes are useful in the detection, identification and isolation of particular gene sequences or products. A probe can also be an antibody that binds an antigen or epitope in a target. A probe is often labeled so that it is detectable in a detection system, such as, for example and without limitation, an enzyme (*e.g.*, ELISA, as well as enzyme-based histochemical assays), fluorescent, radioactive, or luminescent detection system.

"Recombinant" refers to the results of methods, reagents, and laboratory manipulations in which nucleic acids or other biological molecules are enzymatically, chemically or biologically cleaved, synthesized, combined, or otherwise manipulated *in vitro* to produce desired products in cells or other biological systems. For example, a polynucleotide can be inserted into a suitable vector, such as a bacterial plasmid, and the plasmid can be used to transform a suitable host cell to produce a recombinant protein encoded by the polynucleotide. The transformed host cell may be a prokaryotic or eukaryotic, including a bacterial, yeast, insect, or mammalian, cell. Thus, "recombinant DNA" refers to a DNA molecule that is produced by such techniques, and a "recombinant host cell" refers to a cell that comprises a recombinant nucleic acid molecule, typically a recombinant plasmid or other expression vector. Recombinant host cells can express genes that are not found within the native (non-recombinant) form of the cell. For example, such cells can produce a "recombinant protein," which refers to a protein that is produced by expression of a recombinant DNA that encodes the amino acid sequence of the protein.

"Sample" refers to a composition of matter and typically comprises a cell extract, cell, tissue, or a compound or composition derived therefrom, such as a chromosome, genomic DNA, RNA, cDNA, and the like.

"Senescent gene expression" refers to the expression of genes and gene products that are differentially expressed in a senescent as opposed to a young cell. Senescent gene expression can be altered by increasing the expression of young cell specific genes and/or decreasing expression of senescent cell specific genes. These cell specific genes are also denoted as "senescence-related genes". The proteins encoded by the senescence-related genes are also referred to herein as "senescence-related proteins."

"Specifically binds to" or "specific binding" or "specifically binding" refers to the ability of one molecule, typically a macromolecule such as an antibody or oligonucleotide, to contact and associate with another specific molecule in the presence of other different molecules. For example, a single-stranded oligonucleotide "specifically bind to" a single-stranded oligonucleotide that is complementary in sequence, and an antibody "specifically binds to" or "is specifically immunoreactive with" its corresponding antigen.

"Specific hybridization" refers to the formation of hybrids between a probe polynucleotide (*e.g.*, a polynucleotide of the invention which may include substitutions, deletions, and/or additions) and a specific target polynucleotide (*e.g.*, a polynucleotide having the sequence of a GC6 gene or gene product) through base pairing, wherein the probe preferentially hybridizes to the specific target and not to other polynucleotides in the mixture that do not share sequence identity with the target.

"Standard growth conditions" refers to the use of standard culture conditions, *i.e.*, physiological condition and medium used to grow or culture cells.

"Substantially pure" means an object species is the predominant species present (*i.e.*, on a molar basis, more abundant than any other individual macromolecular species in the composition), and a substantially purified fraction is a composition wherein the object species typically comprises at least about 50 percent (on a molar basis) of all macromolecular species present. Generally, a substantially pure composition means that about 80 to 90 percent or more of the macromolecular species present in the composition is the purified species of interest. The object species is purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods) if the composition consists essentially of a single macromolecular species. Solvent species, small molecules (<500 Daltons), stabilizers (*e.g.*, BSA), and elemental ion species are typically not considered macromolecular species for purposes of this definition.

"Suitable reaction conditions" refers to those conditions suitable for conducting a specified reaction, typically using commercially available reagents. Such conditions are known or readily established by those of skill in the art for a variety of reactions. For example, suitable polymerase chain reaction (PCR) conditions include those conditions specified in U.S. Patents 4,683,202; 4,683,195; 4,800,159; and 4,965,188, each of which is incorporated herein by reference.

"Target" refers to the region, sequence or object fixed as a point for amplification, binding, synthesis, isolation, or the like. For example, target can refer to the region of nucleic acid bounded by the primers used for polymerase chain reaction, the region of a nucleic acid to which a probe specifically hybridizes, or the region on a peptide or protein to which an antibody binds.

"Therapeutically effective amount or dose" refers to an amount of a pharmaceutical compositions that is administered to a patient suffering from a disease and is sufficient to cure or at least partially arrest or ameliorate the symptoms of the disease and its complications. The amount will depend on the severity of the disease and the patient and can be determined by standard pharmaceutical procedures in cell cultures or experimental animals. The therapeutic index is the ratio LD50/ED50 (ED50, the dose therapeutically effective in 50% of the population; and LD50, the dose lethal to 50% of the population), and pharmaceutical compositions that exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosages for human use. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

II. REAGENTS AND COMPOUNDS OF THE INVENTION

The methods and reagents of the present invention in part arise out of the recognition that the structural and functional changes in organs and tissues that are intrinsic to the aging process can be attributed to an alteration in the pattern of gene expression that accompanies cell senescence. Research into cellular aging has provided insight into the mechanism through which the lifespan of cells is regulated. Hayflick and Moorhead reported in 1961 that, with continuous passage, human diploid fibroblasts reach replicative senescence at a characteristic number of population doublings. Somatic cells derived from the tissues of a young individual and grown in culture can divide a maximum of about 50-100 times before reaching senescence. Furthermore, the upper limit in the number of cell divisions is inversely related to the age of the donor. Replicative senescence is a genetically-programmed series of changes exhibited by normal cells that culminates in exit from the cell cycle and expression of a senescent genotype and phenotype.

As the body ages, the proportion of senescent cells increases. The accumulation of such cells has both direct and indirect effects that contribute to age related changes and pathologies. As a cell becomes senescent, changes in the pattern of gene expression lead to functional changes. These changes can then influence the physiology of surrounding cells by altering the extracellular environment or in a paracrine fashion through the release of different proteins. For instance, the consequence of an accumulation of senescent cells within the skin is a progressive decrease in skin structure and function. Thus, modulation of senescent gene expression can be used to ameliorate the problems associated with the accumulation of senescent cells.

One approach to alter senescent gene expression is through regulation of steady-state mRNA levels at the transcriptional and post-transcriptional level. However, old cells can differ from young cells not only by altered steady state mRNA levels but also by altered levels of a protein or the activity of a protein, which can be due to alterations in mRNA translation or protein structure. Thus, regulation of gene expression can occur by a variety of mechanisms. At the transcriptional level, the production of mRNAs can either increase or decrease. The level of translation or changes in post-translational modification can lead to an increase or decrease in the abundance of proteins. The activity of a protein can be modulated or the turnover rate of the protein can change. Each of these mechanisms can in turn be regulated.

The present invention provides compounds and reagents relating to the GC6 gene, the expression of which is controlled at least in part by the aging process. The present invention provides useful nucleic acids in isolated form derived from the GC6 gene, including its promoter and coding sequence. These nucleic acids are useful as primers and probes in diagnostic methods and as components of recombinant DNA cloning and/or expression vectors, as well as for other applications described herein. The present invention also provides recombinant and synthetic GC6 gene products and antibodies that bind specifically to the pGC6 gene product, as further described below.

The GC6 gene product was identified as a senescence-specific gene by a technique known as Enhanced Differential Display (EDD; Villeponteau *et al.*, U.S. Patent No. 5,580,726, issued Dec. 3, 1996; incorporated herein by reference). In this technique, cDNAs of differentially expressed mRNAs from young and old fibroblast strains are synthesized using 3' (T rich) primers for targeting the poly A tail of pol II mRNA transcripts and 5' arbitrary primers, as described in Linskens *et al.*, 1995, *Nucleic Acids Research 23*:3244-3251 and Villeponteau *et al.*, *supra*; both incorporated herein by reference.

A 3' primer, 5'-GCGCAAGCTTTTTTTTTTTTGG-3' (SEQ ID NO. 1), designated as "E," and a 5' primer, 5'-CGG GAA GCT TAC TCC ATG ACT C-3' (SEQ ID NO. 2), designated as "11" generated a "genetag," designated "11E3," that was cloned and partially sequenced. The sequence obtained, 5'-AGGGGCACAAGAGTTTGCGGTTATTGAATCCTGAGANAA-3' (SEQ ID NO. 3), wherein N indicates that the identity of the nucleotide at that position was not determined), did not match any known sequence.

A probe corresponding to the genetag 11E3 was prepared by restriction enzyme digestion of a plasmid containing the genetag followed by isolation of the appropriate fragment on low-melting agarose and labeling of the fragment using the random hexamer-primed method (Feinberg & Vogelstein, 1983, *Analyt. Biochem. 132*:6-13). Screening of a commercially available human cDNA library using the probe identified a plasmid, designated pGC6L, that comprised a cDNA of about 2.7 kb with an open reading frame (ORF) about 1625 nucleotides in length and encoding approximately 542 amino acids or a predicted gene product of approximately 59.5 kD. Because

Northern analysis of fibroblast RNA with probes corresponding to the 11E3 genetag and clone identified two mRNA species of 3.3. and 2.7 kb in length, the coding sequence of pGC6L appeared to be incomplete.

A probe was therefore generated by PCR using one primer corresponding to the 5' end of the clone -- this primer was designated KJC47 and is defined by the sequence: 5'-GCAGGAAGGCGGCACGAGAG-3' (SEQ ID NO. 4) -- and another primer corresponding to an internal sequence -- this primer was designated KJC48 and is defined by the sequence: 5'-TTGTATCTTTGTTTGGGATG-3' (SEQ ID NO. 5). The resulting probe was used to screen a lambda GT11 cDNA library prepared using RNA isolated from the 293 HEK cell line.

A lambda clone, designated lambda 3-1-4, was isolated, and PCR using the primer KJC42, defined by the sequence: 5'-TAGGCCCAGATCACTCTCACAGTG CTA T-3' (SEQ ID NO. 6), and a gt11 primer defined by the sequence: 5'-CACCAGACCAACTGGTAATGGTAGCGAC-3' (SEQ ID NO. 7), was used to generate a duplex DNA containing the insert. The PCR product was ligated into pCR2.1 TA cloning vector (Invitrogen) to generating a plasmid designated as pGC6-5'end. DNA sequencing showed that pGC6-5'end contained the remaining coding sequence for the GC6 protein.

The present invention provides recombinant plasmids that can be used to express biologically active pGC6. To prepare such plasmids, the nucleotide sequence encoding the GC6 ORF, or its functional equivalent, is inserted into an appropriate expression vector (*i.e.*, a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence). A GC6 eukaryotic expression vector, pCIneoGC6, comprising the full-coding sequence for GC6 was assembled by splicing the appropriate coding sequences of the pGC6L and lambda 3-1-4 and synthetic and recombinant nucleic acids.

To construct plasmid pCIneoGC6, a nucleic acid comprising the 5' end of the GC6 ORF was generated by amplifying lambda 3-1-4 with primer KJC42 and a primer, KJC51, containing an XhoI site and defined by the sequence:
5'-ATACCGCTCGAGCGGACCTGATTCCCCAGTTGG-3' (SEQ ID NO. 8). This PCR product, designated KJC51-KJC42, is defined by the sequence:

```
5'-  ATACCGCTCG AGCGGACCTG ATTCCCCAGT TGGAATACTC CAGCCCCTTG
     GAAATTCCCG GGATTTATAA AATAACTCTA GACAACAAGA CTTTGTCTTT
     AAAGGTCCTA TGAATTCTTT TCTCTCTGTA TTTANGTATC CTGATTTTTC
     TTTTCCATAT TTTCCACAGG ATTATTTTAC AAATGCAAAT AGAGAGTTGA
     AAAAAGATGC TCAGCAAGAT TACCATCTAG AATATGCCAT GGAAAATAGC
     ACACACACAA TAATTGAATT TACCAGAGAG CTGCATACAT GTGACATAAA
     TGACAAGAGT ATAACGGATA GCACTGTGAG AGTGATCTGG GCCTA-3'
```

(SEQ ID NO. 9), and was ligated into TA cloning vector, pCR2.1 (Invitrogen). A restriction fragment, designated 5'GC6 *XhoI/NcoI*, and defined by the sequence:

```
5'-  TCGAGCGGAC  CTGATTCCCC  AGTTGGAATA  CTCCAGCCCCT TGGAAATTCC
     CGGGATTTAT  AAAATAACTC  TAGACAACAA  GACTTTGTCT  TTAAAGGTCC
     TATGAATTCT  TTTCTCTCTG  TATTTANGTA  TCCTGATTTT  TCTTTTCCAT
     ATTTTCCACA  GGATTATTTT  ACAAATGCAA  ATAGAGAGTT  GAAAAAAGAT
     GCTCAGCAAG  ATTACCATCT  AGAATATGC-3'
```

(SEQ ID NO. 10) was then generated by digesting the resultant plasmid with restriction enzymes *Nco*I and *Xba*I. A restriction fragment, designated GC6 *NcoI/Xba*I, comprising the 3' end fragment was generated by digesting pGC6L with restriction enzymes *Nco*I and *Xba*I. The DNA sequence for GC6 *NcoI/Xba*I is shown below (in the 5' to 3' direction).

CATGGAAAATAGCACACACACAATAATTGAATTTACCAGAGAGCTGCATA
CATGTGACATAAATGACAAGAGTATAACGGATAGCACTGTGAGAGTGATC
TGGGCCTACCACCATGAAGATGCAGGAGAAGCTGGTCCCAAGTACCATGA
CTCCAATAGGGGCACCAAGAGTTTGCGGTTATTGAATCCTGAGAAAACTA
GTGTGCTATCTACAGCCTTACCATACTTTGATCTGGTAAATCAGGACGTC
CCCATCCCAAACAAAGATACAACATATTGGTGCCAAATGTTTAAGATTCC
TGTGTTCCAAGAAAAGCATCATGTAATAAAGGTTGAGCCAGTGATACAGA
GAGGCCATGAGAGTCTGGTGCACCACATCCTGCTCTATCAGTGCAGCAAC
AACTTTAACGACAGCGTTCTGGAGTCCGGCCACGAGTGCTATCACCCCAA
CATGCCCGATGCATTCCTCACCTGTGAAACTGTGATTTTTGCCTGGGCTA
TTGGTGGAGAGGGCTTTTCTTATCCACCTCATGTTGGATTATCCCTTGGC
ACTCCATTAGATCCGCATTATGTGCTCCTAGAAGTCCATTATGATAATCC
CACTTATGAGGAAGGCTTAATAGATAATTCTGGACTGAGGTTATTTTACA
CAATGGATATAAGGAAATATGATGCTGGGGTGATTGAGGCTGGCCTCTGG
GTGAGCCTCTTCCATACCATCCCTCCAGGGATGCCTGAGTTCCAGTCTGA
GGGTCACTGCACTTTGGAGTGCCTGGAAGAGGCTCTGGAAGCCGAAAAGC
CAAGTGGAATTCATGTGTTTGCTGTTCTTCTCCATGCTCACCTGGCTGGC
AGAGGCATCAGGCTGCGTCATTTTCGAAAAGGGAAGGAAATGAAATTACT
TGCCTATGATGATGATTTTGACTTCAATTTCCAGGAGTTTCAGTATCTAA
AGGAAGAACAAACAATCTTACCAGGAGATAACCTAATTACTGAGTGTCGC
TACAACACGAAAGATAGAGCTGAGATGACTTGGGGAGGACTAAGCACCAG
GAGTGAAATGTGTCTCTCATACCTTCTTTATTACCCAAGAATTAATCTTA
CTCGATGTGCAAGTATTCCAGACATTATGGAACAACTTCAGTTCATTGGG
GTTAAGGAGATCTACAGACCAGTCACGACCTGGCCTTTCATTATCAAAAG
TCTCAAGCAATATAAAAACCTTTCTTTCATGGATGCTATGAATAAGTTTA
AATGGACTAAAAAGGAAGGTCTCTCCTTCAACAAGCTGGTCCTCAGCCTG
CCAGTGAATGTGAGATGTTCCAAGACAGACAATGCTGAGTGGTCGATTCA
AGGAATGACAGCATTACCTCCAGATATAGAAAGACCCTATAAAGCAGAAC
CTTTGGTGTGTGGCACGTCTTCTTCCTCTTCCCTGCACAGAGATTTCTCC
ATCAACTTGCTTGTTTGCCTTCTGCTACTCAGCTGCACGCTGAGCACCAA
GAGCTTGTGATCAAAATTCTGTTGGACTTGACAATGTTTTCTATGATCTG
AACCTGTCATTTGAAGTACAGGTTAAAGACTGTGTCCACTTTGGGCATGA
AGAGTGTGGAGACTTTTCTTCCCCATTTTCCCTCCCTCCTTTTTCCTTTC
CATGTTACATGAGAGACATCAATCAGGTTCTCTTCTCTTTCTTAGAAATA
TCTGATGTTATATATACATGGTCAATAAAATAAAACTGGCCTGACTTAAG
ATAACCATTTTAAAAAATTGGGCTGTCATGTGGGAATAAAAGAATTCTTT
CTTTCCTACTACATTCTGTTTTATTTAAATACTCATTGTTGCTATTTCAC
TTTTTGACTTGACTTTTATATTTCTTTAAAAAATTCCTTCCTTTTAAAAA
ATATAAAAGGGACTACTGTTCATTCCAGTTTTCTTCTTCTTTGTTGTTCT
TCTAGTGTGACTTTTCAAGTGTAACAGCCATTCTTCCTGACTTTAATATT
GTCCAGTTCTGGTCTTTTCTGTGAATTACCACTGGGCCCCTTACCTCAAT
GCTTTTTGTTGATGCCCACTCTGGTTCCCTTGTTTATCTGAGTCTGTTGG
TACCCCAAATGACCCCACACCCATYTTAAAGTACTTTTTTTCACCTTCCC
TGTTTAGTACTGGCCAGATGAGTTTTTT

(SEQ ID NO. 11).

These fragments, 5'GC6 *XhoI/Nco*I and GC6 *NcoI/Xba*I, were then ligated into an expression plasmid, designated pCIneo, that had been digested with restriction enzymes *Xho*I and *Xba*I to yield the GC6 expression plasmid pCIneoGC6. The sequence of this vector was

confirmed using sequencing primers KJC52, defined by the sequence: 5'-TAATACGACTCACTATAG-3' (SEQ ID NO. 12); and KJC53, defined by the sequence: 5'-ATTAACCCTCACTAAAGGGA-3' (SEQ ID NO. 13). The DNA sequence for the complete ORF of the GC6 gene, the 5' and 3' untranslated regions of the GC6 mRNA, and the amino acid sequence encoded by the ORF and that defines pGC6 are shown below (nucleic acid sequences are shown in the 5' to 3' direction; amino acid sequences are shown in the amino to carboxy terminus direction; an * designates a stop codon).

```
ACTTTCCAAGGAAGGAAAGGCACACAATGGTATCAAATGTTC   42
ATTCATTCCATCTCTTGATGCTCTACGATATTATCAGTCTAC   84
ACTATGCTTTCCTGAAAGGCCAGAAGTTCAAAGATGGACTAG  126
TTTCCCAGGGACCTGATTCCCCAGTTGGAATACTCCAGCCCC  168
TTGGAAATTCCCGGGATTTATAAAATAACTCTAGACAACAAG  210

                      M  N  S  F  L  S  V  F    8
ACTTTGTCTTTAAAGGTCCTATGAATTCTTTTCTCTCTGTAT  252

   R  Y  P  D  F  S  F  P  Y  F  P  Q  D  Y   22
TTAGGTATCCTGATTTTTCTTTTCCATATTTTCCACAGGATT  294

   F  T  N  A  N  R  E  L  K  K  D  A  Q  Q   36
ATTTTACAAATGCAAATAGAGAGTTGAAAAAAGATGCTCAGC  336

   D  Y  H  L  E  Y  A  M  E  N  S  T  H  T   50
AAGATTACCATCTAGAATATGCCATGGAAAATAGCACACACA  378

   I  I  E  F  T  R  E  L  H  T  C  D  I  N   64
CAATAATTGAATTTACCAGAGAGCTGCATACATGTGACATAA  420

   D  K  S  I  T  D  S  T  V  R  V  I  W  A   78
ATGACAAGAGTATAACGGATAGCACTGTGAGAGTGATCTGGG  462

   Y  H  H  E  D  A  G  E  A  G  P  K  Y  H   92
CCTACCACCATGAAGATGCAGGAGAAGCTGGTCCCAAGTACC  504

   D  S  N  R  G  T  K  S  L  R  L  L  N  P  106
ATGACTCCAATAGGGGCACCAAGAGTTTGCGGTTATTGAATC  546

   E  K  T  S  V  L  S  T  A  L  P  Y  F  D  120
CTGAGAAAACTAGTGTGCTATCTACAGCCTTACCATACTTTG  588

   L  V  N  Q  D  V  P  I  P  N  K  D  T  T  134
ATCTGGTAAATCAGGACGTCCCCATCCCAAACAAAGATACAA  630

   Y  W  C  Q  M  F  K  I  P  V  F  Q  E  K  148
CATATTGGTGCCAAATGTTTAAGATTCCTGTGTTCCAAGAAA  672

   H  H  V  I  K  V  E  P  V  I  Q  R  G  H  162
AGCATCATGTAATAAAGGTTGAGCCAGTGATACAGAGAGGCC  714

   E  S  L  V  H  H  I  L  L  Y  Q  C  S  N  176
ATGAGAGTCTGGTGCACCACATCCTGCTCTATCAGTGCAGCA  756

   N  F  N  D  S  V  L  E  S  G  H  E  C  Y  190
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ACAACTTTAACGACAGCGTTCTGGAGTCCGGCCACGAGTGCT  798

  H  P  N  M  P  D  A  F  L  T  C  E  T  V  204
ATCACCCCAACATGCCCGATGCATTCCTCACCTGTGAAACTG  840

  I  F  A  W  A  I  G  G  E  G  F  S  Y  P  218
TGATTTTTGCCTGGGCTATTGGTGGAGAGGGCTTTTCTTATC  882

  P  H  V  G  L  S  L  G  T  P  L  D  P  H  232
CACCTCATGTTGGATTATCCCTTGGCACTCCATTAGATCCGC  924

  Y  V  L  L  E  V  H  Y  D  N  P  T  Y  E  246
ATTATGTGCTCCTAGAAGTCCATTATGATAATCCCACTTATG  966

  E  G  L  I  D  N  S  G  L  R  L  F  Y  T  260
AGGAAGGCTTAATAGATAATTCTGGACTGAGGTTATTTTACA 1008

  M  D  I  R  K  Y  D  A  G  V  I  E  A  G  274
CAATGGATATAAGGAAATATGATGCTGGGGTGATTGAGGCTG 1050

  L  W  V  S  L  F  H  T  I  P  P  G  M  P  288
GCCTCTGGGTGAGCCTCTTCCATACCATCCCTCCAGGGATGC 1092

  E  F  Q  S  E  G  H  C  T  L  E  C  L  E  302
CTGAGTTCCAGTCTGAGGGTCACTGCACTTTGGAGTGCCTGG 1134

  E  A  L  E  A  E  K  P  S  G  I  H  V  F  316
AAGAGGCTCTGGAAGCCGAAAAGCCAAGTGGAATTCATGTGT 1176

  A  V  L  L  H  A  H  L  A  G  R  G  I  R  330
TTGCTGTTCTTCTCCATGCTCACCTGGCTGGCAGAGGCATCA 1218

  L  R  H  F  R  K  G  K  E  M  K  L  L  A  344
GGCTGCGTCATTTTCGAAAAGGGAAGGAAATGAAATTACTTG 1260

  Y  D  D  D  F  D  F  N  F  Q  E  F  Q  Y  358
CCTATGATGATGATTTTGACTTCAATTTCCAGGAGTTTCAGT 1302

  L  K  E  E  Q  T  I  L  P  G  D  N  L  I  372
ATCTAAAGGAAGAACAAACAATCTTACCAGGAGATAACCTAA 1344

  T  E  C  R  Y  N  T  K  D  R  A  E  M  T  386
TTACTGAGTGTCGCTACAACACGAAAGATAGAGCTGAGATGA 1386

  W  G  G  L  S  T  R  S  E  M  C  L  S  Y  400
CTTGGGGAGGACTAAGCACCAGGAGTGAAATGTGTCTCTCAT 1428

  L  L  Y  Y  P  R  I  N  L  T  R  C  A  S  414
ACCTTCTTTATTACCCAAGAATTAATCTTACTCGATGTGCAA 1470

  I  P  D  I  M  E  Q  L  Q  F  I  G  V  K  428
GTATTCCAGACATTATGGAACAACTTCAGTTCATTGGGGTTA 1512

  E  I  Y  R  P  V  T  T  W  P  F  I  I  K  442
AGGAGATCTACAGACCAGTCACGACCTGGCCTTTCATTATCA 1554
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  S  L  K  Q  Y  K  N  L  S  F  M  D  A  M  456
AAAGTCTCAAGCAATATAAAAACCTTTCTTTCATGGATGCTA 1596

  N  K  F  K  W  T  K  K  E  G  L  S  F  N  470
TGAATAAGTTTAAATGGACTAAAAAGGAAGGTCTCTCCTTCA 1638

  K  L  V  L  S  L  P  V  N  V  R  C  S  K  484
ACAAGCTGGTCCTCAGCCTGCCAGTGAATGTGAGATGTTCCA 1680

  T  D  N  A  E  W  S  I  Q  G  M  T  A  L  498
AGACAGACAATGCTGAGTGGTCGATTCAAGGAATGACAGCAT 1722

  P  P  D  I  E  R  P  Y  K  A  E  P  L  V  512
TACCTCCAGATATAGAAAGACCCTATAAAGCAGAACCTTTGG 1764

  C  G  T  S  S  S  S  S  L  H  R  D  F  S  526
TGTGTGGCACGTCTTCTTCCTCTTCCCTGCACAGAGATTTCT 1806

  I  N  L  L  V  C  L  L  L  L  S  C  T  L  540
CCATCAACTTGCTTGTTTGCCTTCTGCTACTCAGCTGCACGC 1848

  S  T  K  S  L  *                          545
TGAGCACCAAGAGCTTGTGATCAAAATTCTGTTGGACTTGAC 1890

AATGTTTTCTATGATCTGAACCTGTCATTTGAAGTACAGGTT 1932
AAAGACTGTGTCCACTTTGGGCATGAAGAGTGTGGAGACTTT 1974
TCTTCCCCATTTTCCCTCCCTCCTTTTTCCTTTCCATGTTAC 2016
ATGAGAGACATCAATCAGGTTCTCTTCTCTTTCTTAGAAATA 2058
TCTGATGTTATATATACATGGTCAATAAAATAAAACTGGCCT 2100
GACTTAAGATAACCATTTTAAAAAATTGGGCTGTCATGTGGG 2142
AATAAAAGAATTCTTTCTTTCCTACTACATTCTGTTTTATTT 2184
AAATACTCATTGTTGCTATTTCACTTTTTGACTTGACTTTTA 2226
TATTTCTTTAAAAAATTCCTTCCTTTTAAAAAATATAAAAGG 2268
GACTACTGTTCATTCCAGTTTTCTTCTTCTTTGTTGTTCTTC 2310
TAGTGTGACTTTTCAAGTGTAACAGCCATTCTTCCTGACTTT 2352
AATATTGTCCAGTTCTGGTCTTTTCTGTGAATTACCACTGGG 2394
CCCCTTACCTCAATGCTTTTTGTTGATGCCCACTCTGGTTCC 2436
CTTGTTTATCTGAGTCTGTTGGTACCCCAAATGACCCCACAC 2478
CCATYTTAAAGTACTTTTTTTCACCTTCCCTGTTTAGTACTG 2520
GCCAGATGAGTTTTTTCTAGAGCTCTGTCACTATCTGAAAAG 2562
AAAGAGGCTATGGGAAACATAGAAATGGTATGTATTAATAAC 2604
TGATCATAGGCTGAGGAGAAAAAATGTAGCTGGCTGCAAACC 2646
CAGTGCTGTGAGGTGACTTATATGAGGTTCCAGATCAAAGAC 2688
AGGCCGTGTGAGCCAGTCCAGGAGGGTGTAAGTTCTGAATGG 2730
TTCCTTGCTGACTTTGGGTGACACATGTACCACATACTGGCT 2772
CAGTTTAAGTCATGGTTCTATTGTAGATTTATTTTTATATTA 2814
GTTAATAAATGACTTTAAATTGTCACCAATTGAAAATCTTGT 2856
CACTCTTTTGGTTTTCTTTATATAGCTCAGCCAAATCTCTGT 2898
TTTATGTCCTGTCCTCATCTCTTAAGCTAAATCTGTTTGGAT 2940
CATATTAATAAACCTCGTGCCGAATTCGAT              2970
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(SEQ ID NO. 14).

The GC6 ORF encodes a protein of 545 amino acids with a predicted primary translation product size of 622607 Daltons. A comparison of the predicted GC6 protein (pGC6) to human (Genbank accession number P09172), bovine (Genbank accession number P15101), and rat (Genbank accession number Q05754) dopamine beta-hydroxylase (DBH), also known as dopamine monooxygenase precursor, showed that pGC6 has significant homology to these proteins, with many conserved cysteine residues suggesting a similar overall folding conformation. The ORF of the GC6 gene is:

5'-ATGAATTCTTTTCTCTCTGTAT
TTAGGTATCCTGATTTTTCTTTTCCATATTTTCCACAGGATT
ATTTTACAAATGCAAATAGAGAGTTGAAAAAAGATGCTCAGC
AAGATTACCATCTAGAATATGCCATGGAAAATAGCACACACA
CAATAATTGAATTTACCAGAGAGCTGCATACATGTGACATAA
ATGACAAGAGTATAACGGATAGCACTGTGAGAGTGATCTGGG
CCTACCACCATGAAGATGCAGGAGAAGCTGGTCCCAAGTACC
ATGACTCCAATAGGGGCACCAAGAGTTTGCGGTTATTGAATC
CTGAGAAAACTAGTGTGCTATCTACAGCCTTACCATACTTTG
ATCTGGTAAATCAGGACGTCCCCATCCCAAACAAAGATACAA
CATATTGGTGCCAAATGTTTAAGATTCCTGTGTTCCAAGAAA
AGCATCATGTAATAAAGGTTGAGCCAGTGATACAGAGAGGCC
ATGAGAGTCTGGTGCACCACATCCTGCTCTATCAGTGCAGCA
ACAACTTTAACGACAGCGTTCTGGAGTCCGGCCACGAGTGCT
ATCACCCCAACATGCCCGATGCATTCCTCACCTGTGAAACTG
TGATTTTTGCCTGGGCTATTGGTGGAGAGGGCTTTTCTTATC
CACCTCATGTTGGATTATCCCTTGGCACTCCATTAGATCCGC
ATTATGTGCTCCTAGAAGTCCATTATGATAATCCCACTTATG
AGGAAGGCTTAATAGATAATTCTGGACTGAGGTTATTTTACA
CAATGGATATAAGGAAATATGATGCTGGGGTGATTGAGGCTG
GCCTCTGGGTGAGCCTCTTCCATACCATCCCTCCAGGGATGC
CTGAGTTCCAGTCTGAGGGTCACTGCACTTTGGAGTGCCTGG
AAGAGGCTCTGGAAGCCGAAAAGCCAAGTGGAATTCATGTGT
TTGCTGTTCTTCTCCATGCTCACCTGGCTGGCAGAGGCATCA
GGCTGCGTCATTTTCGAAAAGGGAAGGAAATGAAATTACTTG
CCTATGATGATGATTTTGACTTCAATTTCCAGGAGTTTCAGT
ATCTAAAGGAAGAACAAACAATCTTACCAGGAGATAACCTAA
TTACTGAGTGTCGCTACAACACGAAAGATAGAGCTGAGATGA
CTTGGGGAGGACTAAGCACCAGGAGTGAAATGTGTCTCTCAT
ACCTTCTTTATTACCCAAGAATTAATCTTACTCGATGTGCAA
GTATTCCAGACATTATGGAACAACTTCAGTTCATTGGGGTTA
AGGAGATCTACAGACCAGTCACGACCTGGCCTTTCATTATCA
AAAGTCTCAAGCAATATAAAAACCTTTCTTTCATGGATGCTA
TGAATAAGTTTAAATGGACTAAAAAGGAAGGTCTCTCCTTCA
ACAAGCTGGTCCTCAGCCTGCCAGTGAATGTGAGATGTTCCA
AGACAGACAATGCTGAGTGGTCGATTCAAGGAATGACAGCAT
TACCTCCAGATATAGAAAGACCCTATAAAGCAGAACCTTTGG
TGTGTGGCACGTCTTCTTCCTCTTCCCTGCACAGAGATTTCT
CCATCAACTTGCTTGTTTGCCTTCTGCTACTCAGCTGCACGC
TGAGCACCAAGAGCTTG (SEQ ID NO. 15).

The amino acid sequence of pGC6, as predicted from the ORF sequence of the GC6 gene is shown below (in the amino to carboxy terminus direction):

MNSFLSVFRYPDFSFPYFPQDYFTNANRELKKDAQQDYHLEYAMENSTHTIIEFTRELHTCDINDKSITDSTVRVIW
AYHHEDAGEAGPKYHDSNRGTKSLRLLNPEKTSVLSTALPYFDLVNQDVPIPNKDTTYWCQMFKIPVFQEKHHVIKV
EPVIQRGHESLVHHILLYQCSNNFNDSVLESGHECYHPNMPDAFLTCETVIFAWAIGGEGFSYPPHVGLSLGTPLDP
HYVLLEVHYDNPTYEEGLIDNSGLRLFYTMDIRKYDAGVIEAGLWVSLFHTIPPGMPEFQSEGHCTLECLEEALEAE
KPSGIHVFAVLLHAHLAGRGIRLRHFRKGKEMKLLAYDDDFDFNFQEFQYLKEEQTILPGDNLITECRYNTKDRAEM
TWGGLSTRSEMCLSYLLYYPRINLTRCASIPDIMEQLQFIGVKEIYRPVTTWPFIIKSLKQYKNLSFMDAMNKFKWT
KKEGLSFNKLVLSLPVNVRCSKTDNAEWSIQGMTALPPDIERPYKAEPLVCGTSSSSSLHRDFSINLLVCLLLLSCT
LSTKSL (SEQ ID NO. 16).

DBH exists in both the dimeric and tetrameric forms, with two copper atoms per monomeric subunit. The four subunits are linked by disulfide bridges into two dimers, which are joined to each other by noncovalent bonds. Copper, molecular oxygen, and ascorbic acid are all essential for DBH activity. DBH expression is inducible by insulin-like growth factor-I (IGF-I), and DBH functions in catacholamine synthesis by converting dopamine to norepinephrine. Norepinephrine is a critical determinant, along with epinephrine, of minute-to-minute neural regulation of local vascular tone and arterial pressure. DBH is involved in the regulation of autonomic outflow at the level of the brain stem and spinal cord and therefore, contemporaneously has a significant influence on cardiac, renal, and vascular function. In the periphery, the effects of norepinephrine generally result in the elevation of blood pressure, and any factor that alters the synthesis of norepinephrine can perturb blood pressure regulation. The inhibition of DBH in rats elicits a dose-dependent decrease in mean arterial blood pressure (Ohlstein *et al.*, 1987 *J. Pharm. Exp. Ther. 241*:554-559).

Analysis of the pGC6 amino acid sequence shows that pGC6, unlike DBH, lacks a signal peptide sequence at the amino terminus, suggesting that pGC6 may be localized to the cytoplasm. GC6 gene products are believed to be involved in regulating and modulating the sympathetic nervous system. GC6 gene products are therefore not only indicative of cellular senescence but also of other diseases or conditions associated with adrenergic dysfunction and aging, such as hypertension and phenochromocytoma.

The present invention provides synthetic and recombinant oligonucleotides and nucleic acids relating to the GC6 gene in a variety of forms, *i.e.*, isolatable, isolated, purified, substantially pure, or incorporated into a cell-free transcription and translation system or a recombinant host cell. Such compounds are useful for a variety of purposes, *i.e.*, as probes or primers, as polynucleotide plasmids and vectors for producing recombinant gene products that alter senescent gene expression in host cells, as restriction fragments useful for creating useful nucleic acids, and as reagents for therapeutic, diagnostic, and other applications. In particular, the invention provides recombinant or synthetic nucleic acids comprising at least about 8 to 10 to 15 to 25 to 100 or more contiguous nucleotides substantially identical or complementary in sequence to a contiguous nucleotide sequence of the human GC6 gene. One such useful nucleic acid of the invention is the ~3 kb *Xho*I-*Xba*I restriction fragment of plasmid pCIneoGC6.

The novel oligonucleotide probes and primers of the invention typically comprise nucleotides in a sequence substantially identical or complementary to a sequence of nucleotides in a GC6 gene or gene product to allow specific hybridization thereto in a complex mixture of nucleic acids. The oligonucleotide probes and primers of the invention have useful application in a variety of diagnostic, therapeutic, and other applications. The oligonucleotides of the invention can be used as hybridization probes or PCR primers to detect the presence of GC6 gene products, to

diagnose a disease characterized by the presence of an elevated or reduced GC6 mRNA level in cells, to perform tissue typing (*i.e.*, identify tissues characterized by the expression of GC6 mRNA), and the like. Probes can be used to detect GC6-specific nucleotide sequences in a DNA sample, such as for forensic DNA analysis or for diagnosis of diseases characterized by amplification, alteration, and/or rearrangements of the GC6 gene.

The primers and probes of the invention can be used in a variety of diagnostic methods such as to determine relative levels of senescent gene products in tissues. For example, relative levels of GC6 mRNA were determined in various tissues and organs by dot-blot analysis of total RNA (commercially available from Clontech), as described in Example 4, below. The highest levels of GC6 mRNA were detected in fetal and adult kidney, uterus and lung cells. These results were consistent with reverse transcriptase-PCR (RT-PCR) analysis using the primers of the invention:
KJC32, defined by the sequence: 5'-AGCCGAAAAGCCAAGTG-3' (SEQ ID NO. 17), and KJC33, defined by the sequence: 5'-CCTCCCCAAGTCATCTCAG-3' (SEQ ID NO. 18), as described in Example 3. This RT-PCR analysis showed a 3 to 6-fold higher expression in lung, kidney, and testis than in small intestine, thymus, fetal liver, liver and heart and that the expression levels of GC6 were 4 to 5-fold higher in late passage, senescent BJ cells than in early passage, young BJ cells.

As one example of other useful oligonucleotides of the invention, antisense polynucleotides targetting the GC6 mRNA are provided. Complementary antisense polynucleotides include antisense RNA or DNA oligonucleotides that can hybridize specifically to GC6 mRNA or the GC6 gene and so prevent either transcription of the gene or translation of the mRNA. Antisense polynucleotides of various lengths may be used, although such antisense polynucleotides typically comprise a sequence of at least about 25 consecutive nucleotides that are substantially identical to a naturally occurring GC6 gene or corresponding mRNA sequence. Antisense polynucleotides may be produced from an expression vector comprising a heterologous promoter operably linked to a sequence encoding the antisense oligonucleotide in a transfectant cell or transgenic cell. Alternatively, the antisense polynucleotides may comprise soluble oligonucleotides that are administered to the external milieu, either in the culture medium *in vitro* or in the circulatory system or interstitial fluid *in vivo*. Soluble antisense polynucleotides present in the external milieu have been shown to gain access to the cytoplasm and inhibit translation of specific mRNA species. In some embodiments, the antisense polynucleotides comprise methylphosphonate or other synthetic moieties. For general methods relavent to the antisense polynucleotides of the invention, see Antisense RNA and DNA , D.A. Melton, Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1988).

Thus, the present invention also provides antisense molecules comprising the nucleic acid sequence complementary to at least a portion of the polynucleotide of SEQ ID NO. 14 or SEQ ID NO. 15. In an alternatively preferred embodiment, the present invention also provides pharmaceutical compositions comprising an antisense molecules complementary in sequence to a sequence of SEQ ID NO. 14 or SEQ ID NO. 15, and a pharmaceutically acceptable excipient and/or other compound (*e.g.*, adjuvant). Such antisense oligonucleotides have application in reducing transcription of the GC6 gene and translation of GC6 mRNA. These antisense oligonucleotides will be administered to patients and cells in which it is desired to reduce the activity or amount of pGC6. In one embodiment, the antisense oligonucleotides of the invention are administered to alter senescent gene expression by reducing the expression of the GC6 gene via transcription or translation inhibition.

Modulation of GC6 gene expression can be obtained by using the antisense molecules (DNA, RNA, PNA, and the like) of the invention to target the control regions of the GC6 gene (*i.e.*, the promoters, enhancers, and introns). Oligonucleotides derived from the transcription initiation site (*e.g.*, between -10 and +10 regions of the mRNA) are often preferred. The antisense molecules may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing compromises the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules (for a review of recent therapeutic advances using triplex DNA; see Gee *et al.*, in Huber and Carr, Molecular and Immunologic Approaches, Futura Publishing Co, Mt Kisco NY (1994).

Antisense molecules of the invention may be prepared by a wide variety of methods known in the art. These include techniques for chemically synthesizing oligonucleotides, such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences complementary to either strand of the coding sequence of the GC6 gene. Such DNA sequences may be incorporated into a wide variety of vectors with suitable promoters. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly can be introduced into cell lines, cells or tissues.

The antisense molecules of the invention may be modified to increase intracellular stability and half-life. Such modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. The use of PNAs and the inclusion of nontraditional bases such as inosine, queosine and wybutosine as well as acetyl-, methyl-, thio- and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases can also increase stability.

In accordance with the present invention, polynucleotide sequences which encode pGC6, or its functional equivalents, may be used in recombinant DNA molecules that direct the expression of pGC6 by appropriate host cells. Recombinant DNA techniques can be employed to engineer bacteria and other easily cultured organisms to uptake a recombinant expression vector. Other methods for introducing vectors into cells or tissues include those methods discussed infra, and which are equally suitable for *in vivo*, *in vitro* and *ex vivo* therapy. In addition, delivery by transfection and by liposomes are quite well known in the art.

The nucleic acid reagents of the invention also include reagents useful in identifying, isolating, and cloning nucleic acids that encode mammalian (*i.e.*, mouse) homologs of the human GC6 gene. Homologous DNA can be readily identified by screening a genomic or cDNA clone library prepared from the mammalian cells of interest, such as a mouse, rat, rabbit, or other cells, *i.e.*, in yeast artificial chromosomes, cosmids, or bacteriophage lambda (*e.g.*, Charon 35), with a polynucleotide probe comprising a sequence of about at least 15 to 30 or more contiguous nucleotides (or their complement) of the human GC6 sequences disclosed herein. Typically, hybridization and washing conditions are performed at varying degrees of stringency according to conventional hybridization procedures. Positive clones are isolated and sequenced. For illustration and not limitation, a full length polynucleotide corresponding to the open reading frame sequences of the GC6 genes can be labeled and used as a hybridization probe to isolate genomic clones from a murine or other mammalian genomic clone or cDNA library (*i.e.*, those available from Promega Corporation, Madison, Wisconsin).

The nucleic acids of the invention can also be employed to isolate and identify gene products that interact with or bind to GC6 gene products. The yeast "two-hybrid" system (see

Chien *et al.*, 1991, *Proc. Natl. Acad. Sci. 88*:9578) utilizes expression vectors that encode the predetermined polypeptide sequence as a fusion protein and is used to identify protein-protein interactions *in vivo* through reconstitution of a transcriptional activator (see Fields and Song, 1989 *Nature 340*:245). Usually the yeast Gal4 transcription protein, which consists of separable domains responsible for DNA-binding and transcriptional activation, serves as the transcriptional activator. Polynucleotides encoding two hybrid proteins, one consisting of the yeast Gal4 DNA-binding domain fused to a polypeptide sequence of a first protein and the other consisting of the Gal4 activation domain fused to a polypeptide sequence of a second protein (either the first or second protein typically is a number of different proteins to be screened for ability to interact specifically with the other protein), are constructed and introduced into a yeast host cell. Intermolecular binding, if any, between the two fusion proteins reconstitutes the Gal4 DNA-binding domain with the Gal4 activation domain, which leads to the transcriptional activation of a reporter gene (*e.g.*, lacZ, HIS3) operably linked to the Gal4 binding site. Typically, the two-hybrid method is used to identify polypeptide sequences that interact with a known protein.

The nucleic acid sequence encoding pGC6 and variants thereof can also be used to generate hybridization probes for mapping the naturally occurring homologous genomic sequence in the human and other genomes. Mapping techniques include *in situ* hybridization to chromosomal spreads, flow-sorted chromosomal preparations, or artificial chromosome constructs such as yeast artificial chromosomes, bacterial artificial chromosomes, bacterial P1 constructs or single chromosome cDNA libraries as reviewed by Price, 1993, *Blood Rev. 7*:127, and Trask, 1991, *Trends Genet. 7*:149), or radiation hybrid panel mapping. Fluorescent *in situ* hybridization (FISH) of chromosome spreads is described in Verma *et al.*, Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York NY (1988). See also, Hudson *et al.*, 1995, *Science 270*:1945.

Correlation between the location of the sequence encoding human pGC6 on a physical chromosomal map and a specific disease (or predisposition to a specific disease) can delimit the region of DNA associated with the disease. The oligonucleotides of the present invention can also be used to detect differences in gene sequences between normal, carrier or affected individuals. Radiation hybrid panel mapping was used to determine the chromosomal location of the GC6 gene. Hybrid panel mapping entails the use of panels of hybrid cell lines established by using various media in which, for example, one species' cells, such as mouse cells, cannot grow but another species' cells, such as human cells, can. Thus, an individual human chromosome can be probed for the presence of a particular gene, for example, by testing a cell line biochemically for a particular enzyme or immunologically (with an antibody) for a surface antigen; or DNA hybridization techniques may be used to locate a particular DNA sequence. Using gene-specific PCR amplification, the GC6 gene is predicted to lie on the q arm of chromosome 6, closest to the marker D6S413 (lod score 10.8).

Another important application of the oligonucleotide and nucleic acid reagents of the invention relates to the production of recombinant peptides and proteins of the invention. The peptides and proteins of the invention can be produced by *in vitro* recombinant DNA techniques, synthetic techniques and *in vivo* recombination or genetic recombination. Such techniques are generally described in Sambrook *et al.*, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview NY (1989), and Ausubel *et al.*, Current Protocols in Molecular Biology, John Wiley & Sons, New York NY (1989).

The expression vectors of the invention typically comprise expression control sequences operatively linked to a nucleotide sequence, the coding sequence, encoding amino acids in a sequence identical to a at least a portion of the sequence of amino acids in pGC6. The coding

sequence typically encodes at least 6 to 10 amino acids, or encodes all of or at least an active portion of the pGC6, or encodes from 6 to 15 to 20 to 25 to 100 or more contiguous amino acids in a sequence of pGC6, *i.e.*, SEQ ID NO. 16, or variant but related sequence.

Useful GC6 variant proteins include fusion proteins, in which all or a portion of pGC6 is fused to peptide or polypeptide that imparts some useful feature, such as a binding site for use in affinity purification, *i.e.*, a polyhistidine tag of about six histidine residues or the maltose binding protein. Preferably, these amino acid sequences occur in the given order of the naturally occurring proteins (in the amino-terminal to carboxy-terminal orientation) but may comprise other intervening and/or terminal sequences; generally such polypeptides are less than about 500 amino acids in length.

These and other expression vectors of the invention have many useful applications, including in therapeutic methods of the invention as gene therapy vectors for modulating senescence in a target cell or tissue. In one embodiment, recombinant pGC6 is produced in a cell to increase the activity or amount of pGC6 in that cell or an organism comprising the cell. The pGC6 produced in this embodiment of the invention has application to the treatment of diseases caused or exacerbated by inadequate production of pGC6. Variants of pGC6 that have pGC6 activity or enhanced, relative to naturally occurring pGC6, activity are also useful in the treatment of such diseases. In another embodiment, a recombinant pGC6 variant that lacks activity or has diminished, relative to naturally occurring pGC6, activity is administered to treat diseases or conditions in which pGC6 activity is causative or contributes to the disease state.

Thus, the gene therapy expression vectors of the invention also include those that encode variants or "muteins" of pGC6, *i.e.*, express proteins that differ from pGC6 by deletion, substitution, and/or addition of one or more amino acids, and the invention also provides such muteins, whether produced by recombinant or synthetic means. As noted above, the gene therapy vectors of the invention may also, however, encode other useful nucleic acids, such as antisense nucleic acids or ribozymes that target GC6 mRNA. The vectors of the invention can also code for the expression of a protein which, when presented as an immunogen, elicits the production of an antibody that specifically binds to pGC6 or cells expressing those proteins. Such antibodies would be desired to treat or prevent diseases characterized by overexpression of pGC6 or where pGC6 contributes to the disease state or condition. Such vectors can also code for a structurally-related protein, such as a pGC6 fragment or analog.

The nucleotide sequence encoding pGC6 is useful when placed in an expression vector for making quantities of protein for therapeutic or other use. The antisense nucleotide sequence of the GC6 gene is useful in vectors designed for gene therapy directed at diseases associated with senescence. Alternatively, pGC6 encoding nucleotide sequences can be used to direct the expression pGC6 in situations where it is desirable to increase the amount of cellular senescence, *i.e.*, when it is desired to facilitate cellular senescence, for example but not limited to, diseases in which the cell type is proliferative to control growth proliferation.

Expression vectors of the invention comprise expression and replication or chromosomal integration signals or elements compatible with the host cell of interest, *i.e.*, sequences that facilitate transcription and translation (expression sequences) of the coding sequences, such that the encoded polypeptide product is produced. Construction of such polynucleotides is generally well known in the art and is described further in Maniatis *et al.*, supra. For example, but not for limitation, such polynucleotides can include a promoter, a transcription termination site (polyadenylation site in eukaryotic expression hosts), a ribosome binding site, and, optionally, an

enhancer for use in eukaryotic expression hosts, and, optionally, sequences necessary for replication or chromosmal integration of a vector.

A typical eukaryotic expression vector or plasmid of the invention includes a polynucleotide sequence encoding a GC6 polypeptide linked downstream (*i.e.*, in translational reading frame orientation) of a promoter such as the HSV, tk, PGK, metallothionein, or any of a wide variety of other promoters suitable for use in mammalian cells, optionally linked to an enhancer and a downstream polyadenylation site (*e.g.*, an SV40 large T Ag poly A addition site). Expression vectors useful for expressing the recombinant pGC6 of this invention include viral vectors derived from viruses such as retroviruses, herpes viruses, vaccinia viruses, adenoviruses and adeno-associated viruses, *i.e.*, for therapeutic methods; plasmid vectors such as pcDNA1 (Invitrogen, San Diego, CA), in which the expression control sequence comprises the CMV promoter; cosmids, and the like. Viral and plasmid vectors are often preferred for transfecting mammalian cells. Expression vectors can be used for delivery of nucleotide sequences (sense or antisense) to the targeted organ, tissue or cell population.

The polynucleotides comprising the full length cDNA sequence and/or its regulatory elements enable sense (Youssoufian and Lodish, 1993, *Mol. Cell. Biol. 13*:98-104) or antisense (Eguchi *et al.*, 1991, *Ann. Rev. Biochem. 60*:631-652) regulation of gene function. Such technology is now well known in the art for other applications, and sense or antisense oligomers, or larger fragments, can be designed from various locations along the coding or control regions of the GC6 gene. In addition, genes encoding pGC6 can be "turned off" or down-regulated by transfecting a cell or tissue with expression vectors that express high levels of a desired pGC6 fragment. Such constructs can flood cells with untranslatable sense or antisense sequences. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until all the pGC6 or GC6 mRNA is rendered non-functional. Transient expression can last from a day to a week or up to a month or more with a non-replicating vector, and such transient expression vectors are useful and provided by the present invention.

Once a recombinant expression vector is contained within a host or host cell, mRNA synthesis and translation of that mRNA can produce large quantities of a desired protein for use in, for example, medicine, agriculture, and research. The protein can also be used for making a variety of reagents. such as peptides, antibodies, and labeled proteins. Likewise, an unlimited amount of the pure gene can be obtained by replicating the vector and extracting the DNA from the host cell. Thus, in a preferred embodiment, the recombinant expression vectors of the invention are contained within a host cell. Other host cells provided by the invention include those that comprise GC6-derived recombinant or synthetic peptides or proteins.

A variety of expression vector/host systems may be utilized to contain and express a GC6 protein-encoding sequence. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (*e.g.*, baculovirus); plant cell systems transfected with virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with bacterial expression vectors (*e.g.*, Ti or pBR322 plasmid); or animal cell systems.

The "control elements" or "regulatory sequences" of these systems vary in their strength and specificity and include enhancers, promoters, and 3' untranslated regions, which interact with host cellular proteins to carry out transcription and translation. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, inducible

promoters such as the hybrid lacZ promoter of the Bluescript® phagemid (Stratagene, La Jolla CA) or pSport1 (Gibco BRL) and ptrp-lac hybrids and the like may be used. The baculovirus polyhedrin promoter may be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (*e.g.*, heat shock, RUBISCO; and storage protein genes) or from plant viruses (*e.g.*, viral promoters or leader sequences) may be cloned into the vector. In mammalian cell systems, promoters from the mammalian genes or from mammalian viruses are most appropriate. If it is necessary to generate a cell line that contains multiple copies of the sequence encoding pGC6, vectors based on SV40 or EBV may be used with an appropriate selectable marker.

In bacterial systems, a number of expression vectors may be selected depending upon the intended use. For example, when large quantities of pGC6, or GC6 peptides, are needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified may be desirable. Such vectors include, but are not limited to, the multifunctional *E. coli* cloning and expression vectors such as Bluescript® (Stratagene), in which the sequence encoding pGC6 may be ligated into the vector in frame with sequences for the amino-terminal Met and residues of beta-galactosidase so that a hybrid protein is produced (*e.g.*, pIN vectors; Van Heeke and Schuster, 1989, *J. Biol. Chem. 264*:5503-5509). The pGEX vectors (Promega, Madison WI) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems include heparin, thrombin or factor Xa protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

In the yeast, *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase and PGK may be used. For reviews, see Ausubel *et al.* (*supra*), and Grant *et al.*, 1987, *Meth. Enzymol. 153*:516-544.

In cases where plant expression vectors are used, the expression of a sequence encoding pGC6 may be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV (Briton *et al.*, 1984, *Nature 310*:511-514) may be used alone or in combination with the omega leader sequence from TMV (Takamatsu *et al.*, 1987, *EMBO J. 6*:307-311). Alternatively, plant promoters such as the small subunit of RUBISCO (Coruzzi *et al.*, 1984, *EMBO J. 3*:1671-1680; Broglie *et al.*, 1984, *Science 224*:838-843) or heat shock promoters (Winter and Sinibaldi, 1991, *Results Probl. Cell Differ. 17*:85-105) may be used. These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection (for reviews of such techniques, see Hobbs and Murry, in McGraw Hill Yearbook of Science and Technology McGraw Hill New York NY, pp. 191-196 (1992); or Weissbach, Methods for Plant Molecular Biology, Academic Press, New York NY, pp. 421-463 (1988)).

An alternative expression system which could be used to express pGC6 is an insect system. In one such system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. The sequence encoding the pGC6 sequence of interest may be cloned into a nonessential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of the sequence encoding pGC6 will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses are then used to infect *S. frugiperda* cells or *Trichoplusia* larvae in which the pGC6 sequence is expressed (Smith *et al.*, 1983, *J. Virol. 46*:584; Engelhard *et al.*, 1994, *Proc. Natl. Acad. Sci. 91*:3224-7).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, a sequence encoding pGC6 may be ligated into an adenovirus transcription / translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a nonessential E1 or E3 region of the viral genome will result in a viable virus capable of expressing in infected host cells (Logan and Shenk, 1984, *Proc. Natl. Acad. Sci., 81*:3655-59). In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

Specific initiation signals may also be required for efficient translation of a sequence encoding pGC6. These signals include the ATG initiation codon and adjacent sequences. In cases where the sequence encoding pGC6, its initiation codon and upstream sequences are inserted into an appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only the coding sequence, or a portion thereof, is inserted, exogenous transcriptional control signals including the ATG initiation codon must typically be provided. Furthermore, the initiation codon must be in the correct reading frame to ensure translation of the entire coding sequence. Exogenous transcriptional elements and initiation codons can be of various origins, both natural and synthetic. Efficiency of expression may be enhanced by the inclusion of enhancers appropriate to the cell system in use (Scharf *et al.*, 1994, *Results Probl. Cell Differ. 20*:125, and Bittner *et al.*, 1987, *Meth. Enzymol. 153*:516).

In addition, a host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be important for correct insertion, folding and/or function. Host cells, such as CHO (ATCC: American Type Culture Collection, Rockville, MD, CCL 61 and CRL 9618), HeLa (ATCC CCL 2), MDCK (ATCC CCL 34 and CRL 6253), HEK 293 (ATCC CRL 1573), WI-38 (ATCC CCL 75), have specific cellular machinery and characteristic mechanisms for such post-translational activities and may be chosen to ensure the correct modification and processing of the introduced protein.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express pGC6 may be transformed using expression vectors which contain viral origins of replication or endogenous expression elements and a selectable marker gene. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are exposed to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clumps of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus tk or thymidine kinase (Wigler *et al.*, 1977, *Cell 11*:223-32) and aprt or adenine phosphoribosyltransferase (Lowy *et al.*, 1980, *Cell 22*:817) genes which can be employed in tk^- or $aprt^-$ cells, respectively. Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection; for example, dhfr which confers resistance to methotrexate (Wigler *et al.*, 1980, *Proc. Natl. Acad. Sci. 77*:3567); npt, which confers resistance to the aminoglycosides neomycin and G-418 (Colbere-Garapin *et al.*, 1981, *J. Mol. Biol. 150*:1); and als or pat, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murry, In McGraw Hill Yearbook of Science and Technology, McGraw Hill, New York NY, pp 191-196 (1992)), can be employed. Additional

selectable genes have been described, for example, trpB, which allows cells to utilize indole in place of tryptophan, or hisD, which allows cells to utilize histinol in place of histidine (Hartman and Mulligan, 1988, *Proc. Natl. Acad. Sci. 85*:8047). Recently, the use of visible markers has gained popularity with such markers as anthocyanins, beta-glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, being widely used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes *et al.*, 1995, *Meth. Mol. Biol. 55*:121).

The recombinant host cells of the invention have application in many useful methods of the invention. For example, the invention provides recombinant host cells for use in screens to identify agents that alter senescent gene expression, as well as for a variety of other purposes described more fully below. The recombinant host cells of the invention can also be incorporated into the germ line and/or somatic tissues of transgenic mammals, as well as be administered to mammals for therapeutic purposes.

Thus, genomic clones of a senescent-related gene such as the human GC6 gene, or recombinant versions thereof, including versions that encode mutein GC6 gene products, may be used to construct homologous targeting constructs for generating cells and transgenic nonhuman animals having at least one functionally disrupted (or otherwise altered) allele. Guidance for construction of homologous targeting constructs may be found in the art, including: Rahemtulla *et al.*, 1991, *Nature 353*:180; Jasin *et al.*, 1990, *Genes Devel. 4*:157; Koh *et al.*, 1992, *Science 256*:1210; Molina *et al.*, 1992, *Nature 357*:161; Grusby *et al.*, 1991, *Science 253*:1417; and Bradley *et al.*, 1992, *Bio/Technology 10*:534. See also U.S. Patent Nos. 5,464,764 and 5,487,992. Transgenic cells and/or transgenic non-human animals may be used to screen for agents that alter senescent gene expression. Homologous targeting can be used to generate so-called "knockout" mice, which are heterozygous or homozygous for an inactivated allele. Such knockout mice or other mammals may be sold commercially as research animals for investigation of senescence or other purposes.

Chimeric transgenic mice are derived according to Hogan *et al.*, 1988 Manipulating the Mouse Embryo: A Laboratory Manual, Cold Spring Harbor Laboratory, and Teratocarcinomas and Embryonic Stem Cells: A Practical Approach, E.J. Robertson, ed., IRL Press, Washington, D.C. (1987). Embryonic stem cells are manipulated according to published procedures (PCT patent publication No. 96/22362; Zjilstra *et al.*, 1989, *Nature 342*:435; and Schwartzberg *et al.*, 1989, *Science 246*:799, each of which is incorporated herein by reference).

Additionally, a GC6 cDNA or genomic clone may be used to construct transgenes for expressing polypeptides at high levels and/or under the transcriptional control of transcription control sequences which do not naturally occur adjacent to the gene (or vice-versa, *i.e.*, the promoter of the GC6 gene is positioned in front of a reporter gene for use in screening or other use). For example, but not limitation, a constitutive promoter (*e.g.*, an HSV-tk or pgk (phosphoglycerate kinase) promoter) or a cell-lineage specific transcriptional regulatory sequence (*e.g.*, an CD4 or CD8 gene promoter/enhancer) may be operably linked to a protein encoding polynucleotide sequence to form a transgene (typically in combination with a selectable marker such as a neo gene expression cassette). Such transgenes can be introduced into cells (*e.g.*, ES cells, hematopoietic stem cells, cancer cells), and transgenic cells, cell lines, and transgenic animals may be obtained according to conventional methods therewith.

A variety of methods of purifying recombinantly produced pGC6, variant proteins, and peptides derived from either can be used in accordance with the present invention. In addition, such compounds can be produced by wholly synthetic means. For example, host cells transformed

with a nucleotide sequence encoding pGC6 may be cultured under conditions suitable for the expression and recovery of the encoded protein from cell culture. The protein produced by a recombinant cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing pGC6 encoding sequence can be designed with signal sequences which direct secretion of pGC6 through a prokaryotic or eukaryotic cell membrane. Other recombinant constructions may join the sequence encoding pGC6 to a nucleotide sequence encoding a heterologous polypeptide.

The pGC6 or related variant or peptide may also be expressed as a recombinant protein with one or more additional polypeptide domains added to facilitate protein purification. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as polyhistidine tracts and histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp, Seattle WA). The inclusion of a cleavable linker sequences such as Factor Xa or enterokinase (Invitrogen, San Diego CA) between the purification domain and pGC6 is useful to facilitate purification. One such expression vector of the invention provides for expression of a fusion protein comprising the sequence encoding pGC6 and nucleic acid sequence encoding 6 histidine residues followed by thioredoxin and an enterokinase cleavage site. The histidine residues facilitate purification while the enterokinase cleavage site provides a means for purifying pGC6 from the fusion protein. Literature pertaining to vectors containing fusion proteins is available in the art (see *e.g.*, Kroll *et al.*, 1993, *DNA Cell. Biol. 12*:441-53).

Thus, the present invention also provides methods for producing polypeptides comprising the amino acid sequence of SEQ ID NO. 16. This method comprises the steps of: culturing a host cell under conditions suitable for the expression of the polypeptide of SEQ ID NO. 16; and recovering the polypeptide from the host cell culture.

In addition to recombinant production, fragments of pGC6 may be produced by direct peptide synthesis using solid-phase techniques (See *e.g.*, Merrifield, 1963, *J. Am. Chem. Soc. 85*:2149). *In vitro* protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer, Foster City CA) in accordance with the instructions provided by the manufacturer. Various fragments of pGC6 may be chemically synthesized separately and combined using chemical methods to produce the full length molecule.

The newly synthesized peptide can be substantially purified by preparative high performance liquid chromatography (*e.g.*, Creighton, Proteins, Structures and Molecular Principles, WH Freeman and Co, New York NY (1983)). The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (*e.g.*, the Edman degradation procedure; Creighton, *supra*). Additionally the amino acid sequences of pGC6, or any part thereof, may be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

The protein may also have deletions, insertions, or substitutions of amino acid residues that result in a functionally equivalent pGC6 unit. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as the biological activity of the pGC6 is retained, if such activity is desired. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged

polar head groups having similar hydrophilicity values include leucine, isoleucine, valine; glycine, alanine; asparagine, glutamine; serine, threonine; and phenylalanine, tyrosine.

The present invention provides the peptides and proteins encoded by the GC6 gene, as well as fragments and analogs thereof, in isolatable form from eukaryotic or prokaryotic host cells expressing recombinant pGC6, or from an *in vitro* translation system, as well as in purified and substantially pure form from synthesis *in vitro* or by purification from recombinant host cells or by purification of the naturally occurring proteins using antibodies or other reagents of the invention. Methods for expression of heterologous proteins in recombinant hosts, chemical synthesis of polypeptides, and *in vitro* translation are well known in the art and are described further in Maniatis *et al.* and Berger and Kimmel, *supra*. Such proteins have application in screens for therapeutic agents, for diagnostic tests, and for other applications.

Because they are expressed differentially between senescent and non-senescent cells, the GC6 gene and gene products serve as markers of cell senescence. Polypeptides having the full or partial amino acid sequence of pGC6 are useful, for example, in the production of antibodies against pGC6 that are useful in the detection of pGC6 in cells.

Useful pGC6 proteins of the invention may include heterologous sequences linked at the amino- or carboxy-terminus, wherein the heterologous sequence(s) confer a functional property to the resultant analog not shared by the native protein. Such analogs are referred to as fusion proteins and for purposes of the present invention typically comprise pGC6 or its analog and an additional peptide or protein moiety. Fusion proteins usefully combine properties of two different polypeptides or proteins, and can be used, for example, to confer a label, such as a polyhistidine polypeptide or a maltose binding protein, useful in affinity isolation of the fusion protein or to protect the fusion protein from degradation inside a cell. The fusion protein may comprise a linker peptide with desired properties, for example, a peptidase site that renders pGC6 or its analog cleavable from the remainder of the fusion protein. The fusion protein can also confer an antigenic epitope to pGC6; antibodies that bind the epitope could then be used to immunoprecipitate the fusion protein for purification or to identify associated proteins.

One such example is the recombinant GS-GC6 fusion protein. This recombinant fusion protein was produced by inserting a restriction fragment from pGC6L into the bacterial expression vector pGEX-5 (Promega) to form the GS-GC6 bacterial fusion protein vector. The DNA sequence and the encoded amino acid sequence of the GS-GC6 bacterial fusion protein vector is shown below (nucleic acid sequences are shown in the 5' to 3' direction; amino acid sequences are shown in the amino to carboxy terminus direction; an * designates a stop codon).

```
AGCTTATCGACTGCACGGTGCACCAATGCTTCTGGCGTCAGGCAGCCATCGGAAGCTGTG      60
GTATGGCTGTGCAGGTCGTAAATCACTGCATAATTCGTGTCGCTCAAGGCGCACTCCCGT     120
TCTGGATAATGTTTTTTGCGCCGACATCATAACGGTTCTGGCAAATATTCTGAAATGAGC     180
TGTTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCA     240

                    M  S  P  I  L  G  Y  W  K  I  K  G  L  V  Q      15
CACAGGAAACAGTATTCATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGC     300

  P  T  R  L  L  L  E  Y  L  E  E  K  Y  E  E  H  L  Y  E  R      35
AACCCACTCGACTTCTTTTGGAATATCTTGAAGAAAAATATGAAGAGCATTTGTATGAGC     360

  D  E  G  D  K  W  R  N  K  K  F  E  L  G  L  E  F  P  N  L      55
GCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGTTTGGAGTTTCCCAATC     420
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  P  Y  Y  I  D  G  D  V  K  L  T  Q  S  M  A  I  I  R  Y  I      75
TTCCTTATTATATTGATGGTGATGTTAAATTAACACAGTCTATGGCCATCATACGTTATA     480

  A  D  K  H  N  M  L  G  G  C  P  K  E  R  A  E  I  S  M  L      95
TAGCTGACAAGCACAACATGTTGGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGC     540

  E  G  A  V  L  D  I  R  Y  G  V  S  R  I  A  Y  S  K  D  F     105
TTGAAGGAGCGGTTTTGGATATTAGATACGGTGTTTCGAGAATTGCATATAGTAAAGACT     600

  E  T  L  K  V  D  F  L  S  K  L  P  E  M  L  K  M  F  E  D     125
TTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAAAATGTTCGAAG     660

  R  L  C  H  K  T  Y  L  N  G  D  H  V  T  H  P  D  F  M  L     145
ATCGTTTATGTCATAAAACATATTTAAATGGTGATCATGTAACCCATCCTGACTTCATGT     720

  Y  D  A  L  D  V  V  L  Y  M  D  P  M  C  L  D  A  F  P  K     165
TGTATGACGCTCTTGATGTTGTTTTATACATGGACCCAATGTGCCTGGATGCGTTCCCAA     780

  L  V  C  F  K  K  R  I  E  A  I  P  Q  I  D  K  Y  L  K  S     185
AATTAGTTTGTTTTAAAAAACGTATTGAAGCTATCCCACAAATTGATAAGTACTTGAAAT     840

  S  K  Y  I  A  W  P  L  Q  G  W  Q  A  T  F  G  G  G  D  H     205
CCAGCAAGTATATAGCATGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTGGCGACC     900

  P  P  K  S  D  L  I  E  G  R  G  I  P  R  N  S  A  R  E  D     225
ATCCTCCAAAATCGGATCTGATCGAAGGTCGTGGGATCCCCAGGAATTCGGCACGAGAGG     960

  Y  F  T  N  A  N  R  E  L  K  K  D  A  Q  Q  D  Y  H  L  E     245
ATTATTTTACAAATGCAAATAGAGAGTTGAAAAAAGATGCTCAGCAAGATTACCATCTAG    1020

  Y  A  M  E  N  S  T  H  T  I  I  E  F  T  R  E  L  H  T  C     265
AATATGCCATGGAAAATAGCACACACACAATAATTGAATTTACCAGAGAGCTGCATACAT    1080

  D  I  N  D  K  S  I  T  D  S  T  V  R  V  I  W  A  Y  H  H     285
GTGACATAAATGACAAGAGTATAACGGATAGCACTGTGAGAGTGATCTGGGCCTACCACC    1140

  E  D  A  G  E  A  G  P  K  Y  H  D  S  N  R  G  T  K  S  L     305
ATGAAGATGCAGGAGAAGCTGGTCCCAAGTACCATGACTCCAATAGGGGCACCAAGAGTT    1200

  R  L  L  N  P  E  K  T  S  V  L  S  T  A  L  P  Y  F  D  L     325
TGCGGTTATTGAATCCTGAGAAAACTAGTGTGCTATCTACAGCCTTACCATACTTTGATC    1260

  V  N  Q  D  V  P  I  P  N  K  D  T  T  Y  W  C  Q  M  F  K     345
TGGTAAATCAGGACGTCCCCATCCCAAACAAAGATACAACATATTGGTGCCAAATGTTTA    1320

  I  P  V  F  Q  E  K  H  H  V  I  K  V  E  P  V  I  Q  R  G     365
AGATTCCTGTGTTCCAAGAAAAGCATCATGTAATAAAGGTTGAGCCAGTGATACAGAGAG    1380

  H  E  S  L  V  H  H  I  L  L  Y  Q  C  S  N  N  F  N  D  S     385
GCCATGAGAGTCTGGTGCACCACATCCTGCTCTATCAGTGCAGCAACAACTTTAACGACA    1440

  V  L  E  S  G  H  E  C  Y  H  P  N  M  P  D  A  F  L  T  C     405
GCGTTCTGGAGTCCGGCCACGAGTGCTATCACCCCAACATGCCCGATGCATTCCTCACCT    1500

  E  T  V  I  F  A  W  A  I  G  G  E  G  F  S  Y  P  P  H  V     425
```

```
GTGAAACTGTGATTTTTGCCTGGGCTATTGGTGGAGAGGGCTTTTCTTATCCACCTCATG    1560

  G  L  S  L  G  T  P  L  D  P  H  Y  V  L  L  E  V  H  Y  D     445
TTGGATTATCCCTTGGCACTCCATTAGATCCGCATTATGTGCTCCTAGAAGTCCATTATG    1620

  N  P  T  Y  E  E  G  L  I  D  N  S  G  L  R  L  F  Y  T  M     465
ATAATCCCACTTATGAGGAAGGCTTAATAGATAATTCTGGACTGAGGTTATTTTACACAA    1680

  D  I  R  K  Y  D  A  G  V  I  E  A  G  L  W  V  S  L  F  H     485
TGGATATAAGGAAATATGATGCTGGGGTGATTGAGGCTGGCCTCTGGGTGAGCCTCTTCC    1740

  T  I  P  P  G  M  P  E  F  Q  S  E  G  H  C  T  L  E  C  L     505
ATACCATCCCTCCAGGGATGCCTGAGTTCCAGTCTGAGGGTCACTGCACTTTGGAGTGCC    1800

  E  E  A  L  E  A  E  K  P  S  G  I  P  G  S  T  R  A  A  A     525
TGGAAGAGGCTCTGGAAGCCGAAAAGCCAAGTGGAATTCCCGGGTCGACTCGAGCGGCCG    1860

  S  *                                                           526
CATCGTGACTGACTGACGATCTGCCTCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGA    1920
```

(SEQ ID NO. 19). The resulting plasmids were introduced into *E. coli* host bacterial strains, and the cultures were analyzed for the presence of recombinant GS-GC6 fusion protein. Bacterial strains were derived that expressed the recombinant GS-GC6 fusion protein and the resultant protein was purified. The amino acid sequence of the pGS-GC6 fusion protein of the invention is shown below in the amino to carboxy terminus direction.

```
MSPILGYWKIKGLVQPTRLLLEYLEEKYEE      30
HLYERDEGDKWRNKKFELGLEFPNLPYYID      60
GDVKLTQSMAIIRYIADKHNMLGGCPKERA      90
EISMLEGAVLDIRYGVSRIAYSKDFETLKV     120
DFLSKLPEMLKMFEDRLCHKTYLNGDHVTH     150
PDFMLYDALDVVLYMDPMCLDAFPKLVCFK     180
KRIEAIPQIDKYLKSSKYIAWPLQGWQATF     210
GGGDHPPKSDLIEGRGIPRNSAREDYFTNA     240
NRELKKDAQQDYHLEYAMENSTHTIIEFTR     270
ELHTCDINDKSITDSTVRVIWAYHHEDAGE     300
AGPKYHDSNRGTKSLRLLNPEKTSVLSTAL     330
PYFDLVNQDVPIPNKDTTYWCQMFKIPVFQ     360
EKHHVIKVEPVIQRGHESLVHHILLYQCSN     390
NFNDSVLESGHECYHPNMPDAFLTCETVIF     420
AWAIGGEGFSYPPHVGLSLGTPLDPHYVLL     450
EVHYDNPTYEEGLIDNSGLRLFYTMDIRKY     480
DAGVIEAGLWVSLFHTIPPGMPEFQSEGHC     510
TLECLEEALEAEKPSGIPGSTRAAAS         536
```

(SEQ ID NO. 20).

These and other fusion proteins of the invention can be isolated in accordance with standard procedures and then used to immunize animals, *i.e.*, mouse and rabbits, for the production of polyclonal antisera and monoclonal antibodies, as described in the following section.

The pGC6, analogs, peptides, and polypeptides can also be prepared by chemical synthesis using well known methods. For example, various peptides with amino acid sequences corresponding to sequences of pGC6 can be chemically synthesized *in vitro* and used to generate

antibodies that specifically bind to pGC6. An illustrative peptide of the invention includes the GS-GC6 fusion protein, SEQ. ID NO. 20, which has been chemically synthesized *in vitro* and used to immunize animals to generate antibodies specific for pGC6.

Such peptides may correspond to structural and functional domains identified by comparison of the nucleotide and/or amino acid sequence data of a gene or protein to public or other sequence databases. Computerized comparison methods can be used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. See Proteins, Structures and Molecular Principles, Creighton (ed.), W.H. Freeman and Company, New York (1984), incorporated herein by reference. Methods to identify protein sequences that fold into a known three-dimensional structure are known. See Bowie *et al.*, 1991, *Science 253*:164. Recognized sequence motifs and structural conformations may be used to define structural and functional domains. Computer programs GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package (Genetics Computer Group, 575 Science Dr., Madison, WI) can be used to identify sequences in databases, such as GenBank/EMBL, that have regions of homology. Neural network methods, whether implemented in hardware or software, may be used to: (1) identify related protein sequences and nucleotide sequences, and (2) define structural or functional domains in polypeptides. See Brunak *et al.*, 1991, *J. Mol. Biol. 220*:49, incorporated herein by reference.

One important application of the peptides and proteins of the invention is the generation of antibodies that specifically bind to pGC6. The proteins and peptides of the invention can be used to generate antibodies specific for pGC6, or for particular epitopes on those proteins. The pGC6, fragments thereof, or analogs thereof, can be used to immunize an animal for the production of specific antibodies. For the production of antibodies, various hosts, including goats, rabbits, rats, and mice, may be immunized by injection with pGC6 or any portion, fragment or oligopeptide which retains immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants are commercially available, and include but are not limited to Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. BCG (*Bacillus* Calmette-Guerin) and *Corynebacterium parvum* are potentially useful adjuvants.

Monoclonal antibodies to pGC6 can be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include but are not limited to the hybridoma technique originally described by Koehler and Milstein, 1975, *Nature 256*:495-497, the human B-cell hybridoma technique (Kosbor *et al.*, 1983, *Immunol. Today 4*:72; Cote *et al.*, 1983, *Proc. Natl. Acad. Sci. 80*:2026-2030) and the EBV-hybridoma technique (Cole *et al.*, Monoclonal Antibodies and Cancer Therapy, Alan R Liss Inc., New York NY, pp. 77-96 (1985)). Antibodies may also be produced by inducing *in vivo* production in the lymphocyte population or by screening recombinant immunoglobulin libraries or panels of highly specific binding reagents as disclosed in Orlandi *et al.*, 1989, *Proc. Natl. Acad. Sci. 86*: 3833; and Winter and Milstein, 1991, *Nature 349*:293.

For example and without limitation, a recombinantly produced fragment of a pGC6 fusion protein was injected into a rabbit along with an adjuvant following immunization protocols known to those of skill in the art so as to generate an immune response. Specifically, the purified recombinant GS-GC6 fusion protein was injected into rabbits (BABCO) and the resulting serum was tested for reactivity against the fusion proteins by Western blot analysis. Antiserum to the protein was determined to be sensitive and specific.

Alternatively, or in combination with a recombinantly produced polypeptide, a chemically synthesized peptide having an amino acid sequence corresponding to a pGC6 may be used as an immunogen to raise antibodies which bind a pGC6. Immunoglobulins that bind the target protein with a binding affinity of at least about 1 x 10^6 M^{-1} can be harvested from the immunized animal as an antiserum, and may be further purified by immunoaffinity chromatography or other means.

Additionally, spleen cells can be harvested from the immunized animal (typically rat or mouse) and fused to myeloma cells to produce a bank of monoclonal antibody-secreting hybridoma cells. The bank of hybridomas can be screened for clones that secrete immunoglobulins that bind the protein of interest specifically, *i.e.*, with an affinity of at least 1 x 107 M^{-1}. A variety of animals may be used to raise antibodies; for example, mice, rats, goats, rabbits, sheep, and chickens may also be employed to raise antibodies reactive with pGC6. Transgenic animals having the capacity to produce substantially human antibodies also may be immunized and used for a source of antiserum and/or for making monoclonal antibody secreting hybridomas.

Thus, the invention provides polyclonal and monoclonal antibodies that specifically bind to pGC6. In particular, the present invention also provides antibodies that binds specifically to a polypeptide comprising at least a portion of the amino acid sequence of SEQ ID NO. 16 or SEQ ID NO. 20. In one embodiment, the present invention provides a pharmaceutical composition comprising at least one antibody, and a pharmaceutically acceptable excipient.

Bacteriophage antibody display libraries may also be screened for phage able to bind peptides and proteins of the invention specifically. Combinatorial libraries of antibodies have been generated in bacteriophage lambda expression systems and may be screened as bacteriophage plaques or as colonies of lysogens. For general methods to prepare antibodies, see Antibodies: A Laboratory Manual, E. Harlow and D. Lane, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1988), incorporated herein by reference.

These antibodies can in turn be used to isolate pGC6 from normal or recombinant cells and so can be used to purify the proteins as well as other proteins associated therewith. Antibodies directed against pGC6 are useful in the diagnosis and treatment of conditions and diseases associated with senescence. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, Fab fragments and fragments produced by a Fab expression library. These antibodies are useful in the detection of pGC6 in samples and in the detection of cells comprising pGC6 in complex mixtures of cells. Such detection methods have application in screening, diagnosing, and monitoring diseases and other conditions associated with aging, such as hypertension, particularly as pGC6 exhibits significant homology to DBH.

For some applications of the antibodies of the invention, such as identifying immuno-crossreactive proteins, the desired antiserum or monoclonal antibody(ies) is/are not monospecific. In these or other instances, it may be preferable to use a synthetic or recombinant fragment of a pGC6 as an antigen rather than the entire protein. More specifically, where the object is to identify immuno-crossreactive polypeptides that comprise a particular structural moiety, it is preferable to use as an antigen a fragment corresponding to part or all of a commensurate structural domain in pGC6.

Cationized or lipidized antibodies reactive with pGC6 can be used therapeutically to treat or prevent diseases of excessive or inappropriate expression of these proteins and the processes regulated thereby. Other methods of the invention are discussed in the following section.

III. METHODS TO IDENTIFY AGENTS THAT MODULATE SENESCENT GENE EXPRESSION

One important use of the oligonucleotide and antibody probes of the present invention is in a method for screening compounds to identify compounds that can alter senescent gene expression, which method comprises: (a) contacting said cells with an agent; (b) measuring an amount of a GC6 gene product of said treated cells; (c) comparing said measured amount of said GC6 gene product with a measured amount of said GC6 gene product of a control cell not contacted with said agent; and (d) identifying as agents that alter senescent gene expression in cells as any agent that produces an increased or decreased amount of said GC6 gene product in said treated cells in relative to said control cells.

Any GC6 gene product can be used in the method; for example, GC6 mRNA or pGC6. In addition, the assay or screen can be conducted in combination with analysis of the affect of the agent on other known senescent specific markers such as beta-galactosidase, collagenase, interferon gamma, collagen I, collagen III, elastase, elastin, TIMP3, or IL-Ia, autofluorescence, acridine-orange fluorescence, and telomere length. Thus, while one marker can be used to identify agents that alter senescent gene expression, a preferred method is to use a combination of markers.

This screening method identifies compounds with the capacity to reverse, partially reverse, inhibit, or enhance gene expression that is altered as a consequence of senescence. The present invention also encompasses the compounds identified by this method and the use of those compounds to alter gene expression in senescent cells. Such screening can also identify compounds that activate young-specific genes or prevent cells from entering a senescent state. In this method, the oligonucleotide or antibody probes of the invention can serve as indicators of whether a test compound can alter the expression levels of a senescence-related gene.

Compounds ideally suited for testing in this method include compounds identified in primary screens based on other senescent specific markers. However, such testing for other markers can take place before or after screening for agents that modulate GC6 expression. In general, the basic format of the screen is as follows. Cells are cultured in 96-well microtiter plates. After an incubation period, *i.e.*, three days in culture, the medium will be removed and the cells can optionally be assayed for one or more senescence-specific markers, providing a "before treatment" baseline, if desired. The medium will be replaced with fresh medium containing a test agent or its vehicle. The cells will be cultured for an additional period, *i.e.*, two to four days or more in culture, in the presence of the test agent. The cells and/or medium will then be assayed for GC6 gene products ("after treatment" measurement) and compared to non-treated controls.

Compounds found active in the above described screens can then be tested to determine whether the compound inhibits the expression of other senescence-related, specifically old-related, genes or activates the expression of young-related genes, or both. The method can employ Northern analysis to examine the effects of the lead compounds on panels of genes that show altered expression or abundance in senescence. Based on the results of this screen, one can determine which compounds normalize the expression of those genes that are altered in senescence and contribute to age-related pathologies. Furthermore, it will be possible to determine the level at which the compound acts to reverse the pattern of altered expression. Complete reversal to a young pattern of gene expression would suggest that a single common mechanism is involved. Reversal of defined groups of genes would be indicate that several mechanisms are operating and that each is affecting a different set of genes. A compound may also act to modulate the activity of individual genes. This information will in turn influence primary screening strategy. If, for example, all active compounds seem to reverse the altered expression of batteries of genes, or of only

individual genes, then the screen can be expanded so that many more markers, including members from each of the putative batteries, if appropriate, are included.

Cell-based screens have traditionally been labor intensive and so have not often been used for high-throughput screening. However, the present method is amenable to high-throughput screening. Liquid handling operations can be performed by a Microlab 2000™ pipetting station (Hamilton Instruments). Other equipment needed for the screen (*e.g.*, incubators, plate washers, plate readers) can either be adapted for automated functioning or purchased as automated modules. Movement of samples through the assay can be performed by an XPTM robot mounted on a 3 m-long track (Zymark).

In addition, pGC6 or its catalytic or immunogenic fragments or oligopeptides thereof can be used for screening therapeutic compounds in any of a variety of other drug screening techniques. In particular, the GC6 gene product is a useful target for therapeutic intervention because that gene product is involved in disease pathology and a change in its expression parallels that of gene products involved in disease pathology. One can quantitate changes in the level of gene expression caused by a compound using high-throughput screening techniques. The GC6 gene product or fragment thereof employed in such a test may be free in solution, affixed to a solid support, born on a cell surface, or located intracellularly. The formation of binding complexes, between the gene product and the agent being tested, may be measured.

One such screening method uses in situ hybridization to quantitate the expression levels of mRNA before and after treatment of the cells with an agent. Labeled RNA or DNA that is complementary to a specific mRNA, *e.g.*, GC6 mRNA, is prepared. Cells or tissue slices are briefly exposed to heat or acid, which fixes the cell contents, including the mRNA, in place on a glass slide, the fixed cell or tissue is then exposed to the labeled complementary RNA for hybridization. Removal of unhybridized labeled RNA and coating the slide with a photographic emulsion is followed by autoradiogaphy to reveal the presence and even the location of specific mRNA within individual cells.

Alternatively, the amount of mRNA in a sample can be measured and quantitated by competition hybridization. In this method, a measured sample of a specific labeled RNA is exposed to just enough complementary DNA to completely hybridize with it, and a sample of unlabeled RNA is then added. If the unlabeled RNA sample contains the same sequence as the labeled RNA, they compete for the DNA, increasing the ratio of unlabeled to labeled samples decreases the amount of labeled RNA hybridized. The extent to which this takes place is a measure of the amount of competing RNA in the unlabeled sample. Using active agents (agents previously determined to alter expression of senescent genes other that GC6), one can determine whether and at what level coordinate modulation of gene expression occurs (*i.e.*, does the compound affect senescence-related gene globally, in groups, or individually), and if by group, to which group an individual gene belongs.

Another technique for drug screening which may be used for high throughput screening of compounds having suitable binding affinity to the pGC6 is described in detail for other applications in "Determination of Amino Acid Sequence Antigenicity" by Geysen, PCT Application 84/03564, published on September 13, 1984, incorporated herein by reference. In summary, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with fragments of pGC6 and washed. Bound pGC6 is then detected by methods well known in the art. Substantially purified pGC6 can also be coated directly onto plates for use in the aforementioned

drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

Through these screens, libraries of synthetic organic compounds, natural products, peptides, and oligonucleotides can be evaluated for their capacity to alter senescent gene expression that contributes to the disease process. Specifically, compounds can be identified that will down-regulate genes that are up-regulated during senescence or, conversely, will increase the expression of genes that are down-regulated during senescence. Active compounds can be optimized, if desired, via medicinal chemistry. Initially, one can define a pharmacophore(s) using modern computational chemistry tools representative of the structures found to be active in the high throughput screens. Once a consensus pharmacophore is identified, one can design focused combinatorial libraries of compounds to probe structure-activity relationships. Finally, one can improve the biopharmaceutical properties, such as potency and efficacy, of a set of lead structures to identify suitable compounds for clinical testing.

IV. METHODS TO IDENTIFY, OR DISTINGUISH BETWEEN, SENESCENT AND NON-SENESCENT CELLS

In another aspect, the present invention provides diagnostic methods for identifying or distinguishing between senescent and young (also designated herein as quiescent, presenescent, or non-senescent) cells in tissues or in culture. One such method comprises contacting a GC6 gene product within a cell or tissue with an agent that binds specifically to said GC6 gene product under conditions such that said agent and said GC6 gene product bind to one another; determining whether specific binding has occurred; and correlating the presence of senescent and non-senescent cells with the occurrence of binding.

The present invention further provides methods for the detection of pGC6 in a biological sample comprising the steps of: providing a biological sample suspected of expressing human pGC6; and at least one antibody that binds specifically to at least a portion of the amino acid sequence of SEQ ID NO. 16 or SEQ ID NO. 20; combining the biological sample and antibody(ies) under conditions such that an antibody:protein complex is formed; and detecting the complex, wherein the presence of the complex correlates with the expression of the protein in the biological sample. Thus, the present invention provides novel methods and reagents for identifying senescent cells in tissue or culture, which methods generally comprise determining whether a cell expresses a senescence-related gene product, which can include a mRNA or other RNA or a protein, and correlating the presence of that gene product with the state of senescence of the cell or tissue.

Typically, such methods will be practiced using oligonucleotide probe hybridization to the mRNA of the cell, or antibody binding to pGC6 protein either in situ or in a cell extract, quantitating the amount of hybridization or binding, and comparing that amount to a standard or control, such as the amount observed in an untreated young or senescent cell. In one such method, probes specific for the mRNA corresponding to a senescence-related gene are immobilized on a membrane or filter. Then, the cells of interest are cultured under conditions conducive to gene expression and flash-frozen. The cells are then thawed in the presence of a labeled mRNA or protein precursor, so that the label is incorporated into transcripts or proteins that were being transcribed or translated when the cells were frozen. The labeled mRNA or protein is then harvested from the cell and hybridized or bound to the immobilized probes on the filter. The pattern of hybridization or binding will identify whether senescence-related genes are being expressed by the cell. Probes, as described above, specific for GC6 gene products can be used or

other probes specific for GC6 gene products can be made by one of skill in the art as provided by the present invention.

The present invention also provides methods for detecting the presence of nucleotide sequences encoding at least a portion of pGC6 in a biological sample, comprising the steps of, providing: a biological sample suspected of containing nucleic acid corresponding to the nucleotide sequence set forth in SEQ ID NO. 14 or SEQ ID NO. 15; the polynucleotide of SEQ ID NO. 14 or SEQ ID NO. 15 or fragment(s) thereof; combining the biological sample with the polynucleotide under conditions such that a hybridization complex is formed between the nucleic acid and the nucleotide; and detecting the hybridization complex.

In one embodiment of the method the nucleic acid corresponding to the nucleotide sequence of SEQ ID NO. 14 or SEQ ID NO. 15 is ribonucleic acid, while in an alternative embodiment, the nucleotide sequence is deoxyribonucleic acid. In yet another embodiment of the method the detected hybridization complex correlates with expression of the polynucleotide of SEQ ID NO. 14 or SEQ ID NO. 15 in the biological sample. Other identification methods of the invention and diagnostic applications are described below.

The expression of pGC6 in cell lines can be used as a diagnostic for identifying senescent cells and is useful in the diagnosis of diseases associated with senescence and aging. The oligonucleotides of the invention may be used in hybridization protocols to diagnose the induced expression of messenger RNA sequences early in the disease process. Such techniques often employ signal or target amplification, such as PCR, LCR, TAS, 3SR, b-DNA, tyramide signal amplification, and the like. Likewise the protein can be used to produce antibodies useful in ELISA assays or a derivative diagnostic format. Such diagnostic tests allow different classes of senescent cells that exacerbate or induce diseases associated with aging to be distinguished and thereby facilitate the selection of appropriate treatment regimens.

The methods of the invention are especially useful in conjunction with therapeutic regimes and strategies. For example, one aspect of the invention is to provide methods for detecting polynucleotide sequences, including genomic sequences encoding variant pGC6 proteins, to identify the senescent (or young) cells in a tissue sample or to identify cells that produce a disease-causing pGC6 variant protein. The specificity of the probe, whether it is made from a highly specific region (*e.g.*, 10 unique nucleotides in the 5' regulatory region), or a less specific region (*e.g.*, especially in the 3' region), and the stringency of the hybridization or amplification (maximal, high, intermediate or low) will determine whether the probe identifies only naturally occurring pGC6 or variant sequences.

By appropriately labeling the probe for detection (*e.g.*, with a fluorescent molecule or a molecule that serves as a binding partner for another molecule), one can label the senescent (or young) cells in a tissue and then separate the young from the senescent cells. In addition, a detectable reagent (*e.g.*, a contrast molecule for magnetic resonance imaging) can be attached to an antibody or other substance which is specific for a GC6 senescence-related gene product, and can specifically label senescent cells. With such a preparation of cells enriched for either the young or senescent phenotype, one can then perform many useful procedures, including reintroduction of young cells into a host or treatment of senescent cells for reintroduction to the host.

Polynucleotide sequences encoding pGC6 also may be used for the diagnosis of conditions or diseases with which the abnormal expression of pGC6 is associated. For example, polynucleotide sequences encoding human pGC6 may be used in hybridization or PCR assays of fluids or tissues from biopsies to detect pGC6 expression. Such qualitative or quantitative methods

may include Southern or Northern analysis, dot blot or other membrane-based technologies; PCR technologies; dip stick, pin, chip and ELISA technologies. All of these techniques are well known in the art for other purposes and are the basis of many commercially available diagnostic kits.

The oligonucleotide or antibody probe may be labeled by methods known in the art, such as by a variety of reporter groups, including commercially available radionucleotides such as ^{32}P or ^{35}S, or enzymatic labels such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like, and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization or binding complexes. After an incubation period, the sample is washed with a compatible fluid which optionally contains a dye (or other label requiring a developer) if the probe has been labeled with an enzyme. After the compatible fluid is rinsed off, the dye is quantitated and compared with a standard. If the amount of dye in the biopsied or extracted sample is significantly elevated over that of a comparable control sample, the probe has bound to GC6 gene products in the sample, and the presence of elevated levels of the gene product in the sample indicates the presence of the associated disease. Alternatively, the loss of expression of GC6 gene products in a tissue which normally expresses them indicates the reversal of senescence or an associated disease state.

Particular pGC6 antibodies are useful for the diagnosis of conditions or diseases characterized by expression of pGC6, or in assays to monitor patients being treated with agonists or inhibitors (including antisense transcripts) of GC6 gene expression. Diagnostic assays for GC6 gene expression include methods utilizing the antibody and a label to detect pGC6 in human body fluids or extracts of cells or tissues. The polypeptides and antibodies of the present invention may be used with or without modification. Frequently, the polypeptides and antibodies will be labeled by joining them, either covalently or noncovalently, with a reporter molecule. A wide variety of reporter molecules are known, several of which were described above. In particular, the present invention is useful for diagnosis of human disease, although it is contemplated that the present invention will find use in the veterinary arena as well.

A variety of protocols for measuring pGC6 using either polyclonal or monoclonal antibodies specific for the respective protein are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and fluorescent activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on pGC6 is preferred, but a competitive binding assay may be employed. These assays are described, among other places, in Maddox *et al.*, 1983, *J. Exp. Med.* *158*:1211.

Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regime in animal studies, in clinical trials, or in monitoring the treatment of an individual patient. To provide a basis for the diagnosis of disease, a normal or standard profile for human pGC6 expression is usually established. This is accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with pGC6, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained for normal subjects with a dilution series of human pGC6 run in the same experiment where a known amount of substantially purified human pGC6 is used. Standard values obtained from normal samples may be compared with values obtained from samples from patients affected by senescence induced or exacerbated diseases. Deviation between standard and subject values can establish the presence of a disease state. In addition, the deviation can indicate, within a disease state, a particular clinical outcome.

Once disease is established, a therapeutic agent is administered and a treatment profile is generated. Such assays may be repeated on a regular basis to evaluate whether the values in the profile progress toward or return to the normal or standard pattern. Successive treatment profiles may be used to show the efficacy of treatment over a period of several days or several months.

Additionally, methods which may be used to quantitate the expression of a particular molecule include radiolabeling (Melby *et al.*, 1993, *J. Immunol. Meth. 159*:235-44) or biotinylating (Duplaa *et al.*, 1993, *Analyt. Biochem. 229*-36) nucleotides, co-amplification of a control nucleic acid, and standard curves onto which the experimental results are interpolated. Quantitation of multiple samples may be speeded up by running the assay in an ELISA format where the oligomer of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantitation. A definitive diagnosis of this type may allow health professionals to begin aggressive treatment and prevent further worsening of the condition.

V. THERAPEUTIC APPLICATIONS AND PHARMACEUTICAL COMPOSITIONS

In another aspect of the invention the oligonucleotides, proteins, antibodies, and pGC6 agonists, antagonists, or inhibitors, are employed to inhibit or reverse senescent gene expression and to treat age-related disease induced or exacerbated by cellular senescence. Those of skill in the art will recognize that the senescence-related genes and gene products of the invention provide a wide array of such agents that can be used to target or direct therapeutic reagents to young or senescent cells. In one embodiment, antisense oligonucleotides, can be targeted to senescence-specific genes. Such antisense oligonucleotides can be comprised of ribonucleic acids, deoxyribonucleic acids, modified nucleic acids, or mixtures.

In addition, it will be appreciated that therapeutic benefits from treatment of a disease or disease condition induced or exacerbated by senescence can be realized by combining an agent of the invention which alters the senescent genotype or phenotype with other agents. Thus, in its therapeutic applications, the present invention also relates to pharmaceutical compositions which may comprise GC6 nucleotides, proteins, antibodies, agonists, antagonists, or inhibitors, alone or in combination with at least one other agent such as another drug or a stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. Any of these molecules can be administered to a patient alone, or in combination with other agents, drugs or hormones, in pharmaceutical compositions where it is mixed with suitable excipient(s), adjuvants, and/or pharmaceutically acceptable carriers.

The choice of such combinations will depend on various factors including, but not limited to, the type of disease, the age and general health of the patient, the aggressiveness of disease progression, and the ability of the patient to tolerate the agents that comprise the combination. In addition, in some cases it may be advisable to combine a modulating agent of senescent gene expression of the invention with one or more agents that treat the side effects of a disease, *e.g.*, an analgesic, or agents effective to stimulate the patient's own immune response (*e.g.*, colony stimulating factor).

In another embodiment, the present invention includes compounds and compositions in which a modulating agent of the senescent phenotype is either combined with or covalently bound to a cytotoxic agent bound to a targeting agent, such as a monoclonal antibody (*e.g.*, a murine or humanized monoclonal antibody). It will be appreciated that the latter combination may allow the introduction of cytotoxic agents into diseased cells with greater specificity. Thus, the active form of the cytotoxic agent (*i.e.*, the free form) will be present only in cells targeted by the antibody. Of

course, the modulating agents of the senescent phenotype of the invention may also be combined with monoclonal antibodies that have therapeutic activity against pGC6.

In general, a suitable effective does of a compound of the invention will be in the range of 0.001 to 1000 milligram (mg) per kilogram (kg) of body weight of the recipient per day, preferably in the range of 0.001 to 100 mg per kg of body weight per day, more preferably between about 0.1 and 100 mg per kg of body weight per day and still more preferably in the range of between 0.1 to 10 mg per kg of body weight per day. The desired dosage is preferably presented in one, two, three, four, or more subdoses administered at appropriate intervals throughout the day. These subdoses can be administered as unit dosage form, for example, containing 5 to 10,000 mg, preferably 10 to 1000 mg of active ingredient per unit dosage form. Preferably, the dosage is present once per day at a dosing at least equal to TID.

The exact dosage is chosen by the individual physician in view of the patient to be treated. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Additional factors which may be taken into account include the severity of the disease state (*e.g.*, location of the disease, age, weight, and gender of the patient, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy). Long acting pharmaceutical compositions might be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular formulation. General guidance as to particular dosages and methods of delivery for other applications is provided in the literature (see US Patent Nos. 4,657,760; 5,206,344; and 5,225,212, herein incorporated by reference). Those skilled in the art will typically employ different formulations for oligonucleotides and gene therapy vectors than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, and the like.

The compositions used in these therapies can be in a variety of forms. These include, for example, solid, semi-solid, and liquid dosage forms, such as tablets, pills, powders, liquid solutions or suspensions, liposomes, and injectable and infusible solutions. The preferred form depends on the intended mode of administration and therapeutic application. The compositions also preferably include conventional pharmaceutically acceptable carriers and adjuvants, as is well known to those of skill in the art. See, *e.g.*, Remington's Pharmaceutical Science, Mack Publishing Co.; Easton, PA, 17th Ed. (1985). Preferably, administration will be by oral or parenteral (including subcutaneous, intramuscular, intravenous, and intradermal) routes. More preferably, the route of administration will be oral. The therapeutic methods and agents of this invention can of course be used concomitantly or in combination with other methods and agents for treating a particular disease or disease condition.

While it is possible to administer the active ingredient of this invention alone, it is preferable to present a therapeutic agent as part of a pharmaceutical formulation or composition. The formulations of the present invention comprise at least one agent which alters the GC6 senescent genotype of this invention in a therapeutically or pharmaceutically effective dose together with one or more pharmaceutically or therapeutically acceptable carriers and optionally other therapeutic ingredients. Various considerations for preparing such formulations are described, *e.g.*, in Goodman and Gilman's Pharmacological Bases of Therapeutics, 8th Ed., Pergamon Press (1990); and Remington's *supra*, each of which is incorporated herein by reference. Methods for administration are discussed therein, *e.g.*, for oral, intravenous, intraperitoneal, intramuscular, and other forms of administration.

Typically, methods for administering pharmaceutical compositions will be either topical, parenteral, or oral. Oral administration is a preferred. The pharmaceutical compositions can be administered in a variety of unit dosage forms depending upon the method of administration. As noted above, unit dosage forms suitable for oral administration include powders, tablets, pills, and capsules.

One can use topical administration to deliver a compound of the invention by percutaneous passage of the drug into the systemic circulation of the patient. The skin sites include anatomic regions for transdermally administering the drug, such as the forearm, abdomen, chest, back, buttock, and mastoidal area. The compound is administered to the skin by placing on the skin either a topical formulation comprising the compound or a transdermal drug delivery device that administers the compound. In either embodiment, the delivery vehicle is designed, shaped, sized, and adapted for easy placement and comfortable retention on the skin.

A variety of transdermal drug delivery devices can be employed with the compounds of this invention. For example, a simple adhesive patch comprising a backing material and an acrylate adhesive can be prepared. The drug and any penetration enhancer can be formulated into the adhesive casting solution. The adhesive casting solution can be placed directly onto the backing material or can be applied to the skin to form an adherent coating. See, *e.g.*, U.S. Patent Nos. 4,310,509; 4,560,555; and 4,542,012.

In other embodiments, the compounds of the invention will be delivered using a liquid reservoir system drug delivery device. These systems typically comprise a backing material, a membrane, an acrylate based adhesive, and a release liner. The membrane is sealed to the backing to form a reservoir. The drug or compound and any vehicles, enhancers, stabilizers, gelling agents, and the like are then incorporated into the reservoir. See, *e.g.*, U.S. Patent Nos. 4,597,961; 4,485,097; 4,608,249; 4,5005,891; 3,843,480; 3,948,262; 3,053,255; and 3,993,073.

Matrix patches comprising a backing, a drug/penetration enhancer matrix, a membrane, and an adhesive can also be employed to deliver a compound of the invention transdermally. The matrix material typically will comprise a polyurethane foam. The drug, and enhancers, vehicles, stabilizers, and the like are combined with the foam precursors. The foam is allowed to cure to produce a tack, elastomeric matrix which can be directly affixed to the backing material. See, *e.g.*, U.S. Patent Nos. 4,542,013; 4,460,562; 4,466,953; 4,482,543; and 4,533,540.

Also included within the invention are preparations for topical application to the skin comprising a compound of the invention, typically in concentrations in the range from about 0.001% to 10%, together with a non-toxic, pharmaceutically acceptable topical carrier. These topical preparations can be prepared by combining an active ingredient according to this invention with conventional pharmaceutical diluents and carriers commonly used in topical dry, liquid, and cream formulations. Ointment and cremes may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Such bases may include water and/or an oil, such as liquid paraffin or a vegetable oil, such as peanut oil or castor oil. Thickening agents that may be used according to the nature of the base include soft paraffin, aluminum stearate, cetostearyl alcohol, propylene glycol, polyethylene glycols, woolfat, hydrogenated lanolin, beeswax, and the like.

Lotions may be formulated with an aqueous or oily base and will, in general, also include one or more of the following: stabilizing agents, emulsifying agents, dispersing agents, suspending agents, thickening agents, coloring agents, perfumes, and the like. Powders may be

formed with the aid of any suitable powder base, *e.g.*, talc, lactose, starch, and the like. Drops may be formulated with an aqueous base or non-aqueous base also comprising one or more dispersing agents, suspending agents, solubilizing agents, and the like. Topical administration of compounds of the invention may also be preferred for treating diseases associated with the skin.

The topical pharmaceutical compositions according to this invention may also include one or more preservatives or bacteriostatic agents, *e.g.*, methyl hydroxybenzoate, propyl hydroxybenzoate, chlorocreosol, benzalkonium chlorides, and the like. The topical pharmaceutical compositions also can contain other active ingredients such as antimicrobial agents, particularly antibiotics, anesthetics, analgesics, and antipruritic agents.

The compounds or agents of the present invention can also be delivered through mucosal membranes. Transmucosal (*i.e.*, sublingual, buccal, and vaginal) drug delivery provides for an efficient entry of active substances to systemic circulation and reduces immediate metabolism by the liver and intestinal wall flora. Transmucosal drug dosage forms (*e.g.*, tablet, suppository, ointment, pessary, membrane, and powder) are typically held in contact with the mucosal membrane and disintegrate and/or dissolve rapidly to allow immediate systemic absorption. Note that certain such routes may be used even where the patient is unable to ingest a treatment composition orally. Note also that where delivery of a senescent modulating agent of the invention would be enhanced, one can select a composition for delivery to a mucosal membrane.

For delivery to the buccal or sublingual membranes, typically an oral formulation, such as a lozenge, tablet, or capsule, will be used. The method of manufacture of these formulations is known in the art, including, but not limited to, the addition of the pharmacological agent to a pre-manufactured tablet; cold compression of an inert filler, a binder, and either a pharmacological agent or a substance containing the agent (as described in U.S. Patent No. 4,806,356); and encapsulation. Another oral formulation is one that can be applied with an adhesive, such as the cellulose derivative hydroxypropyl cellulose, to the oral mucosa, for example, as described in U.S. Patent No. 4,940,587. This buccal adhesive formulation, when applied to the buccal mucosa, allows for controlled release of the pharmacological agent into the mouth and through the buccal mucosa.

Parenteral administration is generally characterized by injection, either subcutaneously, intramuscularly, or intravenously. Thus, this invention provides compositions for intravenous administration that comprise a solution of a compound of the invention dissolved or suspended in an acceptable carrier. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solutions or suspension in liquid prior to injection, or as emulsions. Suitable excipients are, for example, water, buffered water saline, dextrose, glycerol, ethanol, or the like. These compositions will be sterilized by conventional, well know sterilization techniques, such as sterile filtration. The resulting solutions can be packaged for use as is or lyophilized, the lyophilized preparation being combined with a sterile solution prior to administration. In addition, if desired, the pharmaceutical compositions to be administered may also contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, pH buffering agents and the like, such as, for example, sodium acetate, sorbitan monolaurate, and triethanolamine oleate.

Another method of parenteral administration employs the implantation of a slow-release or sustained-release system, such that a constant level of dosage is maintained. See, *e.g.*, U.S. Patent No. 3,710,795, incorporated herein by reference.

Liquid pharmaceutically administrable compositions can, for example, be prepared by dissolving, dispersing, etc., an active compound as defined above and optional pharmaceutical adjuvants in an excipient, such as, for example, water, saline, aqueous dextrose, glycerol, ethanol, olive oil, and other lipophilic solvents, and the like, to form a solution or suspension. If desired, the pharmaceutical composition to be administered may also contain minor amounts of nontoxic auxiliary substances, such as wetting or emulsifying agents, pH buffering agents, and the like, for example, sodium acetate, sorbitan monolaurate, triethanolamine sodium acetate, triethanolamine oleate, etc. Actual methods of preparing such dosages forms are known and will be apparent to those skilled in this art; for example, see Remington's, supra. The composition or formulation to be administered will contain an effective amount of an active compound of the invention.

For solid compositions, conventional nontoxic solid carriers can be used and include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers previously listed, and generally 0.1-95% of the active ingredient, preferably about 20%.

The compositions containing the compounds of the invention can be administered for prophylactic and/or therapeutic treatments. In therapeutic applications, compositions are administered to a patient already suffering from a disease, as described above, in an amount sufficient to cure or at least partially arrest the symptoms of the disease and its complications. An amount adequate to accomplish this is defined as a "therapeutically effective amount or dose." Therapeutic efficacy and toxicity of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals (*e.g.*, ED50, the dose therapeutically effective in 50% of the population; and LD50, the dose lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

In addition to internal (*in vivo*) administration, the compounds and compositions of the invention may be applied *ex vivo* to achieve therapeutic effects, as, for example, in the case of a patient suffering from AIDS. In such an application, cells to be treated, *e.g.*, blood or bone marrow cells, are removed from a patient and treated with a pharmaceutically effective amount of a compound of the invention. The cells are returned to the patient following treatment. Such a procedure can allow for exposure of cells to concentrations of therapeutic agents for longer periods or at higher concentration than otherwise available.

Once improvement of the patient's conditions has occurred, a maintenance dose is administered, if necessary. Subsequently, the dosage or the frequency of administration, or both, can be reduced, as a function of the systems, to a level at which the improved condition is retained. When the symptoms have been alleviated to the desired level, treatment can cease. Patients can, however, require additional treatment upon any recurrence of the disease symptoms.

In prophylactic applications, compositions containing the compounds of the invention are administered to a patient susceptible to or otherwise at risk of a particular disease. Such an amount

is defined to be a "prophylactically effective amount or dose." In this use, the precise amounts again depend on the patient's state of health and weight.

As will be apparent to those of skill in the art upon reading of this disclosure, the present invention provides valuable methods, compounds, and compositions relating to cellular senescence. It is contemplated that these compounds, for example, antisense molecules capable of reducing the expression of GC6 gene products can be used as therapeutic molecules to treat age-related diseases associated with the expression of GC6. Likewise, antibodies directed against GC6 and capable of neutralizing the biological activity of this gene also may be used as therapeutic molecules to treat age-related diseases such as, for example, hypertension. Thus, the above description of necessity provides a limited and merely illustrative sampling of specific methods, compounds, and compositions and should not be construed as limiting the scope of the invention. Other features and advantages of the invention will be apparent from the following examples and claims.

EXAMPLES

The following examples are provided to demonstrate and further illustrate certain preferred embodiments and aspects of the present invention and are not to be construed as limiting the scope thereof.

In the experimental disclosure which follows, the following abbreviations apply: eq (equivalents); M (Molar); μM (micromolar); N (Normal); mol (moles); mmol (millimoles); μmol (micromoles); nmol (nanomoles); g (grams); mg (milligrams); mg (micrograms); ng (nanograms); l or L (liters); ml (milliliters); ml (microliters); cm (centimeters); mm (millimeters); μm (micrometers); nm (nanometers); °C (degrees Centigrade); dNTP (deoxyribonucleotide); dH2O (distilled water); DDT (dithiothreitol); PMSF (phenylmethylsulfonyl fluoride); TE (10 mM Tris HCl, 1 mM EDTA, approximately pH 7.2); KGlu (potassium glutamate); SSC (salt and sodium citrate buffer); SDS (sodium dodecyl sulfate); and PAGE (polyacrylamide gel electrophoresis). Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are described.

EXAMPLE 1
STANDARD METHODS IN MOLECULAR GENETICS

Standard techniques are used for recombinant nucleic acid methods, polynucleotide synthesis, *in vitro* polypeptide synthesis, microbial culture and transformation (*e.g.*, electroporation), and the like. Generally enzymatic reactions and purification steps using commercially available starting materials are performed according to the manufacturer's specifications. The techniques and procedures are generally performed according to conventional methods in the art and various general references (see, generally, Sambrook *et al.* Molecular Cloning: A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989), incorporated herein by reference) referenced herein.

Oligonucleotides can be synthesized on an Applied Bio Systems or other commercially available oligonucleotide synthesizer according to specifications provided by the manufacturer. Polynucleotide primers may be prepared using any suitable method, such as, for example, the phosphotriester and phosphodiester methods, or automated embodiments thereof. In one such automated embodiment, diethylphosphoramidites are used as starting materials and may be synthesized as described by Beaucage *et al.*, 1981 *Tetrahedron Letters* 22:1859, and U.S. Patent No. 4,458,066.

Methods for PCR amplification are known in the art (PCR Technology: Principles and Applications for DNA Amplification, Ed. Erlich, Stockton Press, New York, NY (1989); PCR Protocols: A Guide to Methods and Applications, eds. Innis, Gelfland, Sninsky, and White, Academic Press, San Diego, CA (1990); Mattila *et al.*, 1991, *Nucleic Acids Res. 19*:4967; Eckert and Kunkel, 1991, *PCR Methods and Applications 1*:17; and the U.S. Patents noted above. Optimal PCR and hybridization conditions will vary depending upon the sequence composition and length(s) of the targeting polynucleotide(s) primers and target(s) employed, and the experimental method selected by the practitioner. Various guidelines may be used to select appropriate primer sequences and hybridization conditions (see, Sambrook *et al.*, supra). Generally PCR is carried out in a buffered aqueous solution, preferably at a pH of 7-9, most preferably about 8. The deoxyribonucleoside triphosphates dATP, dCTP, dGTP, and TTP are also added to the synthesis mixture in adequate amounts, and the resulting solution is heated to about 85-100 °C for about 1 to 10 minutes, preferably from 1 to 4 minutes. After this heating period, the solution is allowed to cool to about 20-40 °C, for primer hybridization. To the cooled mixture is added an agent for polymerization, and the reaction is allowed to occur under conditions known in the art. This synthesis reaction may occur at from room temperature up to a temperature just over which the agent for polymerization no longer functions efficiently. Thus, for example, if a heat-labile DNA polymerase is used as the agent for polymerization, the synthesis temperature is generally no greater than about 45°C. The agent for polymerization may be any compound or system that will function to accomplish the synthesis of primer extension products, including enzymes. Suitable enzymes for this purpose include, for example, *E. coli* DNA polymerase I or the Klenow fragment thereof, *Taq* DNA polymerase, and other available DNA polymerases.

The newly synthesized strand and its complementary nucleic acid strand form double-stranded molecules used in the succeeding steps of the process. In the next step, the strands of the double-stranded molecule are separated using any of the procedures described above to provide single-stranded molecules. The steps of strand separation and extension product synthesis can be repeated as often as needed to produce the desired quantity of the specific nucleic acid sequence. The amount of the specific nucleic acid sequence produced will accumulate in an exponential fashion.

Enhanced differential display of subtracted cDNA involves PCR amplification with 5' arbitrary primer(s) and a 3' oligo dT primer with two randomized bases at the 3' end, recovery of bands identified as containing cDNA corresponding to differentially expressed mRNAs, and PCR amplification, sequencing, and/or cloning of the bands identified. Add 1 µl of one 5' primer (20 µM stock) or two 5' primers (half of each) or 1.2 µl of one 5' primer (1 µl) and one 3' primer (0.2 µl) to the tube. Add 1 µl of subtracted DNA to the same tube. To this mixture, add 8 µl of cocktail mix containing 1 µl of 10X PCR buffer for Pfu polymerase (commercially available), 1 µl of dNTP (2.5 mM each), 0.3 mM alpha-^{32}P-dATP, 0.1 µl of *Taq* polymerase, 0.2 µl of *Pfu* polymerase (Stratagene), 0.02 µl of T4 gene 32 protein (Boehringer Mannheim), and 5.38 µl water. Overlay one drop of mineral oil, and PCR amplify for 4 cycles at 94 degrees C for 45 sec., 39 degrees C for 1 min., and 72 degrees C for 1 min., and then 22 cycles at 94 degrees C for 45 sec., 60 degrees C for 1 min., and 72 degrees C for 1 min., with a final extension for 5 min. at 72 degrees C. About 5 µl of formamide/dye is added to the PCR product, and the products are denatured at 95 degrees C for 2-3 min. and loaded onto a prewarmed 6% polyacrylamide sequencing gel, which is run at 1900 to 2000 constant voltage (do not allow current to reach 50 mA) until the xylene cyanol dye is one inch from the bottom of the gel. The gel is dried under vacuum at 80 degrees C for 45 min. and exposed to PhosphorImager™ screen (for notebook record) and/or then to X-ray film at room temperature for one or two days (tape the gel to

the film and punch three holes at the three corner of the gel and film for easy identification of bands).

Differentially expressed gene fragments appear as bands on the screen or film that are present in the lanes on the gel corresponding to the cDNA of the tester cells but present at lower levels or absent from the lanes corresponding to the cDNA of the control lanes. The bands can be recovered from the gel by first aligning the gel with the film or screen (based on the three holes and marks) and then excising the bands of interest with a razor blade and transferring the gel slice to an Eppendorf™ tube. Rinse the razor blade between each cutting operation to avoid cross contamination. To remove the urea and paper backing used with sequencing gels without substantial loss of the desired DNA, add about 900 µl of TE buffer to the tube containing the gel slice, incubate the tube at room temperature for 10 min., and then remove and discard the paper and TE buffer. To prepare a solution of the desired DNA from the gel slice, the gel slice is suspended in 40 µl of TE buffer containing 100 mM NaCl and heated for 10 min. at 95-98 degrees C. The liquid is collected (a short centrifugation collects the liquid at the bottom of the tube) and serves as a source of the desired DNA.

This DNA can be PCR-amplified by placing 1-3 µl of recovered DNA in a 50 µl total reaction volume in a reaction mixture containing 6 µl of total primer(s), 5 µl of 10x PCR buffer for Pfu polymerase, 6 µl of dNTP (2.5 mM each), 0.25 µl of *Taq* polymerase, 0.5 µl of Pfu polymerase, 0.05 µl of T4 gene 32 protein, and water. The PCR is performed for 25 cycles at 94 degrees C for 45 sec., 60 degrees C for 1 min., and 72 degrees C for 1 min., with a 5 min. extension at 72 degrees C at the end of the last cycle. The PCR products can be stored or further processed, *i.e.*, subcloned and sequenced.

EXAMPLE 2
GC6 SENESCENCE-RELATED GENE EXPRESSION SCREEN

To determine the effect of a compound on the expression levels of a GC6 gene product, such as mRNA or pGC6, according to the method of the present invention, cells are seeded at 10,000 to 20,000 cells/plate in DMEM medium plus 10% Bovine Calf Serum (BCS) and grown in a 10 or 15 cm plate. In a preferred embodiment, senescent fibroblast lines derived from human foreskin (BJ cells) are used at Passage Doubling Level (PDL) 92. Other cells, in the appropriate media, can also be used. After 6 hours, the medium is removed and replaced with DMEM plus 0.5% BCS. After 3 days, the medium is replaced with fresh medium. One plate is then incubated with the test compound, and another plate is incubated with the compound test vehicle alone. In a preferred embodiment, 2 µl of agent dissolved in DMSO (1 µM final concentration), or of DMSO alone, are added to 200 µl of medium. Other volumes, vehicles, and compound concentrations can also be used. In addition, mixtures of compounds, rather than single compounds, can be added to the cells.

After four days of incubation, the cells are lysed in GITC (guanidium isothiocyanate), and either mRNA or pGC6 is analyzed. The RNA can analyzed with the senescence-related gene probes of the invention by Northern analysis or by other suitable methods, such as RT-PCR. The results of this analysis will indicate the efficacy of the compound in altering the mRNA expression level on the GC6 senescence-related gene. In, a preferred embodiment, the expression levels of at least one additional, and preferably 3 to 5 to 10 to 20 or more, senescence-related genes will be determined. In a similar manner, quantitative levels of pGC6 can be determined by the use of pGC6 specific antibodies of the invention.

Thus, agents are tested to determine whether a specific agent alters the expression of the young- and old-specific senescence-related genes identified by EDD and in the scientific literature. If a compound has the effect of complete reversal to a young pattern of gene expression, then the compound impacts a single common mechanism driving cell senescence. Reversal of defined groups of genes indicates that several mechanisms are operating in senescence and that different mechanisms can affect different panels of genes. A compound may also act to modulate the activity of an individual gene, suggesting the absence of a common mechanism.

An alternative screen for compounds that alter the expression of senescence-related gene involves the use of a genetic construct comprising a promoter of a senescence-related gene positioned for expression of a coding sequence from a reporter gene, such as an alkaline phosphatase gene, the expression of which can be efficiently and readily monitored. Such a construct would be used to generate stable transfectants in very early passage cells, such as dermal fibroblasts, and then the cells could be used at any stage up to and including senescence to identify agents that up or down-regulate the expression of the reporter gene.

EXAMPLE 3
RT-PCR METHOD TO DETERMINE GC6 MRNA LEVELS IN VARIOUS TISSUES

One can detect GC6 mRNA by an RT-PCR protocol. First, cDNA synthesis is carried out by mixing together, in an appropriate tube: 1 µg total RNA; 1 µg random hexamer or GC6-specific primer; and deionized water to 10 µl. The tube is heated to 95°C for 10 seconds and then placed immediately on ice.

Then, 4 µl of first strand buffer (Promega); 2 µl of 0.1 M DTT; 1 µl of 10 mM dNTPs; 1 µl of RNAse Guard (Pharmacia); and 1 µl of SuperScript II™ Reverse Transcriptase buffer (Promega) are added, and the reaction is incubated at 42°C for 1 hour. The reaction is stopped by heating the mixture to 95°C for 10 minutes.

The PCR reaction is carried out by adding, in an appropriate tube: 1, 3, or 10 µl of the above cDNA reaction mixture; 3 µl of 10x Ampli*Taq*™ buffer (Perkin-Elmer) 1.8 µl of 25 mM $MgCl_2$; 1.5 µl of 10 µM KJC32 primer; 1.5 µl of 10 µM KJC33 primer; 0.3 µl of Ampli*Taq*™ plymerase (Perkin-Elmer); 3 µl of 10x dNTP mix (2 mM each of dGTP, dCTP, and dTTP, 0.2 mM of dATP); 0.3 µl alpha-^{32}P-dATP (3000 Ci/mMol; NEN-Dupont) and deionized water to 30 µl. The tube is then placed in a thermal cycler programmed to perform 25 cycles of 94°C for 10 seconds; 55°C for 10 seconds; and 72°C for 60 seconds.

For comparative purposes, one can perform paralleled reactions in which a gene expressed at a known level or a constitutively expressed gene, *i.e.*, 28S RNA, is amplified; the number of cycles of PCR amplification can be adjusted in such instances, *i.e.*, for 28S RNA, the PCR amplification may be performed for only 7 cycles. Suitable primers for PCR amplification of 28S RNA include primer 28SF, defined by the sequence: 5' GCTAAATACCGGCACGAGAC CGATAG -3' (SEQ ID NO. 21), and 28SR, defined by the sequence: 5' GGTTTCACGCCCTCTTGAACTCTCT C- 3' (SEQ ID NO. 22). About 10 µl of each PCR reaction are then resolved using native 6% polyacrylamide gels, run in 0.6X TBE buffer. After an appropriate time, the gels are dried and exposed to a PhosphorImager™ plate (Molecular Dynamics). The appropriate bands are then identified and quantified using the PhosphorImager quantification software.

Other useful primers of the invention for amplifying or detecting GC6 gene or mRNA include KJC54, defined by the sequence: 5'-GGACCTGATTCCCCAGTTGG (SEQ ID NO. 23); KJC55, defined by the sequence: 5'-AGTACTGGCCAGATGAGTTT (SEQ ID NO. 24); KJC56, defined by the sequence: 5'-TCACACGGCCTGTCTTTGAT (SEQ ID NO. 25); KJC58, defined by the sequence: 5'-CATGCCCAAAGTGGACACAG (SEQ ID NO. 26); and KJC59, defined by the sequence: 5'-GAATTCTTTTCTCTCTGTATTTAGGTATCCTG (SEQ ID NO. 27).

EXAMPLE 4
DOT -BLOT METHOD TO DETERMINE GC6 MRNA LEVELS IN VARIOUS TISSUES

One can also detect GC6 gene products using a dot blot protocol. A dot blot array (Master Blot) of human RNA samples can be obtained from ClonTech. A ^{32}P-labeled probe was prepared using an *Eco*RI restriction enzyme digest product from the GC6 cDNA and was used as a hybridization probe as described in the manufacturer's protocol.

The probe was produced as follows. Approximately 100 ng of an *Eco*RI restriction fragment from pGC6L was prepared. This fragment was labeled with ^{32}P using a Ready-To-Go™ kit (Pharmacia) according to the manufacturer's instructions. The labeling reaction products were then purified using a Sephacryl S400 HR spin column (Pharmacia) as per the manufacturer's instructions.

The purified probe was hybridized to the Master Blot as instructed by the manufacturer. Briefly, the blot was first pre-hybridized at 65°C in an ExpressHyb™ solution containing 1 mg/ml salmon sperm DNA, then hybridized in a similar solution that contains the prepared, denatured probe and Cot-1 human DNA. Following an overnight incubation at 65°C, the blot was washed under stringent solution conditions and then exposed for an appropriate time to a PhosphorImager™ screen (Molecular Dynamics).

EXAMPLE 5
MULTIPLEX PROBING FOR GC6 MRNA TOGETHER WITH OTHER SENESCENCE-SPECIFIC GENE PRODUCTS

In one embodiment, the GC6 mRNA is measured using an array of senescence-specific gene products. High density DNA array technology is useful method used to detect and quantify the levels of specific transcripts in a nucleic acid mixture. In general, these arrays consist of ordered patterns of DNA molecules, called targets, fixed to a solid-phase matrix, typically silicon. These targets may be manufactured by a variety of means. For example, Affymetrix, Inc., produces DNA arrays using a photolithographic technique in which short oligonucleotides are synthesized *in situ*, while Synteni Corp. deposits solutions containing the target DNAs using high density printing techniques.

To prepare a DNA chip using the latter technique, bacterial clones containing the ESTs (nucleic acids encoding expressed sequence "tags") are grown in an appropriate antibiotic-containing medium, and the plasmid DNA in the clones is amplified using PCR and vector-specific primers. A portion of the amplification products are checked on an agarose gel to verify the size and amount of the amplified target. The remainder of the sample is purified and arrayed onto glass slides.

Poly A+ mRNA samples are purified as described below and fluorescently-labeled. These probes are hybridized to the DNA arrays, which are then subsequently washed, and the bound

fluorescence is quantified. Poly(A+) mRNA is isolated from total RNA using poly-dT resin from the Qiagen OligoTex™ isolation kit. There are kits from Qiagen to isolate polyA directly from cell culture, but it is preferred to isolate total RNA first.

To isolate total RNA, 150 mm cell culture plates containing the cells of interest are washed twice with dilute PBS (Ca and Mg free), About 1 ml of 4 M guanidine isothiocyanate (GITC)/25 mM Na acetate, pH6/0.8% mercaptoethanol is added to the 150 mm plate to facilitate cell lysis and extraction of RNA. The resulting mixture is scraped to one side and pippetted into 6 ml of a GITC/Na acetate/mercaptoethanol solution for a total volume of 7 ml. This mixture is layered this onto an Ultraclear 14x89 mm polycarbonate tube (Beckman 34059) that has 4 ml of 5.7 M CsCl2 as a bedding.

These samples are centrifuged at 32K rpm for 20 hours at 16°C in a SW41 swinging bucket rotor using a Beckman XL-80 ultracentrifuge. The supernatant is removed, and the pellet allowed dry for ~10 minutes. The pellet is resuspended in 360 µl of DEPC water (2 x 180 µl suspensions). About 40 µl of 3M Na acetate (pH 6 or 5.2) is added to the mixture together with 1 ml of 100% cold EtOH, and the resulting mixture is centrifuged for 20-30 minutes at 14K rpm under cold room conditions. The ethanol is removed, and the pellet is allowed to dry and resuspended in an appropriate volume of DEPC water. Purity is checked by measuring OD and 260/280 ratios.

Poly-A mRNa is isolated from total mRNA using a Qiagen Oligotex kit. About 1-250 µg of total RNA is dissolved in DEPC water to 250 µl, and then 250 µl of 2X Binding Buffer and 15 µl of the Oligotex polydT resin suspension are added, the contents mixed in a 1.5 ml Eppendorf tube and incubated at 65°C for three minutes. The mixture is allowed to stand at room temperature for 15-20 minutes and then centrifuged at maximal speed on a benchtop centrifuge at room temperature for 2 minutes. The supernatant is discarded, and the pellet washed once with 400 µl of Wash Buffer QW2.

The contents are transferred to a spin column, which is centrifuged at full speed for about 2 minutes, and then transferred to another 1.5 ml microcentrifuge tube, and washed again with 400 µl of QW2 wash buffer and centrifuged again for 2 minutes at maximum speed. The flowthrough is discarded, and the polyA RNA is washed off by adding 20 µl of DEPC water warmed to 70°C. The above procedure is repeated once, and the eluted material contains polyA mRNA. From 100 µg of total RNA from cell culture, about 1-3 µg of polyA RNA are obtained. Purity and quantity are checked by measuring OD 260/280 ratios and 1% agarose/EtBr gel electrophoresis. This material is suitable for labeling and hybridization to a DNA chip.

An illustrative DNA chip for screening in accordance with the present invention is described below. First, the various nucleic acids, defined by Genbank accession and clone numbers are described; then, a protocol for preparing the nucleic acids to be placed on the chip is described. Thus, one panel of preferred nucleic acids is described in tabular form below.

GenBankID	*CloneID*	*Gene or Marker*
w49497	325050	interstitial collagenase precursor
n69322	285780	collagenase 3 precursor
aa034203	471174	stromelysin-3 precursor
aa046659	487394	plasminogen activator inhibitor-1 (PAI-1)

GenBankID	*CloneID*	*Gene or Marker*
H48533	201890	TNFa
aa283090	713145	hyaluronic acid receptor (CD44)
t80285	24664	hyaluronidase
aa293101	726240	collagen 1a1
aa398701	727729	collagen 1a3
t79116	113513	elastin
w88847	417799	fibronectin
aa127854	501773	fibronectin receptor alpha subunit
aa236739	876065	ATM
h68922	212078	integrin alpha subunit (laminin receptor)
aa292578	723936	TIMP-1
aa043969	487021	TIMP-3
h15949	48452	Acid sphingomyelinase
aa029514	366842	N-CAM
aa281410	705220	mortalin
aa284503	713672	prohibitin
aa009762	429771	aldehyde dehydrogenase (ALDH-1)
aa293501	726105	80K-L
aa001357	427894	lipoprotein-associated coagulation inhibitor
aa046260	488870	secretory granule proteoglycan core protein precursor
r21396	36200	human tissue factor
aa058472	489366	IGF binding protein 1
aa040602	376184	IGF binding protein 3
aa029472	366793	IGF binding protein 5
n44809	272210	rho8
r48580	153589	stanniocalcin
t75438	23176	follistatin-related protein
aa215765	683517	GC4
w47081	325063	GC6
w37627	321886	GC7
aa005169	429074	GC10
r01968	124540	Cu/Zn SOD
h65391	210401	MnSOD
aa007652	429455	glutathione synthetase
r46847	152524	p21/SDI1/CIP1/WAF1
w00390	291057	p16
r18972	33368	p27
t89175	110022	cyclin D1
h62385	236338	p53
n25402	264214	Rb susceptibility gene
aa287985	701394	Werner's Syndrome gene
r72795	157725	retinoic acid inducible factor
aa292734	726275	osteonectin
r96180	231021	cyclooxygenase-1 (COX-1)
w93945	357451	EPC-1/PEDF
r08428	127318	ALDH-1 (mitochondrial)

GenBankID	*CloneID*	*Gene or Marker*
H74252	229341	VEGF
w80716	415588	presenilin 1
aa041277	376303	insulin like growth factor(IGF)
H16473	49060	TGF-b1
aa037699	484975	TGF-bbinding protein (endoglin)
w95660	357811	TGF breceptor type 1
r14113	27430	acidic fibroblast growth factor (FGF)
R84654	180204	basic fibroblast growth factor receptor
aa278581	703433	FGF-receptor activating protein I
aa403247	758523	platelet-derived growth factor (PDGF-alpha)
w72000	345645	platelet-derived growth factor (PGDF-beta)
aa054505	489395	PDGF-alpha receptor
aa075724	545136	epidermal growth factor (EGF)
aa026175	469272	EGF receptor
aa207063	682639	TNF a
w72329	345232	TNF beta
w89178	417861	transferrin receptor
aa001614	427812	Insulin receptor
aa009608	365515	keratinocyte growth factor
w74536	346604	RAGE receptor
R17089	129839	protein kinase C zeta
N80314	290412	b-Catenin
r39221	23173	Mitosis activating protein (MAP) kinase
aa278157	712516	MAPKK1
aa425826	769579	MAPKK2 (MEK1/erk activating kinase)
w90037	417357	MAPK phosphatase-1
h87371	252443	MAPK phosphatase-2
N67917	286709	c-fos
W23847	309864	junB
w87741	417226	c-myc
aa058523	489327	c-raf
H61706	206186	c-src
w38444	328467	c-ras
h05603	43504	c-erbA (thyroid hormone receptor)
aa293570	714213	FAS
h74208	232714	BCL-2
t95052	120106	ICE
n76118	299429	ICAM-1
aa281973	712849	Poly (ADP-ribose) polymerase
r17189	32026	DNA-PK
T72581	22040	92 kDa type IV collagenase precursor
T80285	24664	hyaluronidase
T75563	112912	Von Hippel-Lindau Disease tumor suppressor
T79116	113513	elastin
T85543	114712	proteinase 3 precursor
T96150	121022	human monocyte activation antigen (PAI receptor)

GenBankID	*CloneID*	*Gene or Marker*
R01893	124488	soluble epoxide hydrolase
R05275	125077	fibrinogen gamma-B
R42288	30689	proteoglycan link protein precursor
R18972	33368	p27
R52103	40205	cathepsin B
R67275	41676	collagen alpha 1(XI)
R36670	136956	tumor suppressor SNC6
R68482	139242	tumor suppressor, PDGF receptor beta-like
R64102	139584	Rb-binding protein 2
R67214	140827	tumor suppressor DPC4
R79223	146311	72 kDa type IV collagenase precursor (gelatinase A)
R80235	147075	MDM-2
R81706	147787	plakoglobin
R46847	152524	p21/SDI1/CIP1/WAF1
R48414	153530	procollagen-lysine, 2-oxoglutarate 5-dioxygenase precursor
R72075	155716	heregulin
H21471	159990	Cdk2
H08667	45851	collagen, type XVIII, alpha
H15949	48452	acid sphingomyelinase
H15147	49898	APC-1
H49433	178772	Human mRNA for senescence marker protein-30
R83615	187615	calcium-dependent protease, small
H45943	188230	ATM
H48122	193736	BRCA2
R98783	200898	kininogen, LMW precursor
H53852	202647	collagen, type II, alpha 1
H58247	204519	cathepsin E
H58473	205905	glutathione-S-transferase
R98080	206806	tumor suppressor LUCA-1
H61812	236374	Cdk4
N53585	245614	cathepsin S
H81981	249453	tumor suppressor HTS1
N29616	257294	M-phase inducer phosphatase 2
N30878	258129	trichohyalin
N30678	258294	p57KIP2
N25120	262198	beta-catenin
N20106	263118	cartilage homeoprotein 1, CART-1
N62929	278705	Rb susceptibility gene
N58841	288791	proliferation-associated protein PAG (peroxidase antioxidant)
N67639	290753	citrate synthase
N71998	290871	integrin alpha-3 subunit (lam,fibro,collagen receptor)
N72115	291057	p18 (CDK6 inhibitor)
N67818	291622	putative tumor suppressor EXT1
N68071	292115	collagen 1 alpha 2
N80868	300702	thrombospondin 4
W19086	302057	Cockayne's Syndrome gene

GenBankID	*CloneID*	*Gene or Marker*
N93844	309199	collagen XV alpha 1
N93959	309345	membrane-type matrix metalloprotease-2
N94385	309515	thrombospondin 4 (cartilage oligomeric matrix protein)
N94405	309560	collagen III(1
W30775	309591	p300 (E1A binding protein)
W42634	323181	Human fibroblast activation protein mRNA
W44410	323621	growth defect suppressor HCP1 homolog
W47091	324700	stromelysin-1 precursor
W47129	324700	stromelysin-2 precursor
W49497	325050	interstitial collagenase precursor
W49820	325720	probable hyaluronan synthase Has2
W68308	342670	corticosterone methyl oxidase
W68308	342670	brighter band in amp. check comparison of dots.
W67555	343187	HIC-1 tumor suppressor
W68086	343362	paxillin
W73785	344109	PCNA
W73874	345538	cathepsin L
W92603	357681	cathepsin G
W95471	357807	cyclin E
W94563	358017	fibrinogen gamma-A
W94858	358441	growth arrest specific clone 1 (gas1)
AA017532	361203	plasma glutathione peroxidase
AA018325	361518	p19
AA019833	363365	Drosophila tumor-suppressor homolog DLG2
AA025310	364715	thrombospondin 3
AA009519	365465	P-TEN
AA025750	366323	HE4 mRNA for extracellular proteinase inhibitor homologue
AA026625	366539	fibrinogen alpha
AA026137	366639	proteoglycan 2
AA029408	366789	fibromodulin
AA029472	366793	IGF binding protein 5
AA055178	377215	cathepsin C
AA055445	377516	fibrinogen beta
W80458	415495	glutathione peroxidase
W86006	416134	thrombospondin 2
W89129	417320	tissue plasminogen activator precursor
W89002	417469	catalase
AA001329	427857	cyclin A
AA004918	428443	laminin B-1
AA007652	429455	glutathione synthetase
AA009433	429859	ezrin
AA010398	430308	BRCA1-associated RING protein
AA026112	469281	HUGL tumor suppressor
AA029360	470346	laminin B-2
AA031483	470679	collagen IV alpha 1
AA034203	471174	stromelysin-3 precursor

GenBankID	*CloneID*	*Gene or Marker*
AA035118	471736	beta-galactosidase precursor
AA044261	486375	fibronectin receptor beta subunit
AA043969	487021	TIMP-3
AA045473	487887	tenascin
AA045285	487934	vinculin
AA047455	488455	cathepsin D
AA056585	489181	thrombospondin 1
AA056736	489224	Human retinoblastoma-binding protein (RbAp46) mRNA
AA058337	489348	cathepsin H
AA284503	713672	prohibitin
AA284825	713858	aggrecan
AA291692	724729	AT (group D)
aa293101	726240	collagen 1(1
AA393564	727855	glutathione reductase
AA393647	728263	alpha-catenin
AA421151	728610	integrin alpha-6 subunit
AA405362	742884	Cdc2
AA400097	743271	TIMP-2
AA425535	773289	cytochrome C oxidase polypeptide IV
AA454160	795309	extracellular superoxide dismutase precursor
AA453479	795352	focal adhesion kinase-1(FAK-1)
AA461383	796853	cartilage link protein
AA464438	809919	focal adhesion kinase-2 (FAK-2)
T65090	21703	NF2
T89023	22560	1-phosphatidylinositol -4,5-bisphosphate phosphodiesterase beta
T74988	23022	calcium-binding protein BDR-1 (hippocalcin)
R38701	23354	neurite growth-promoting factor 1
R38385	23806	N-cadherin
T77200	23820	aryl hydrocarbon receptor nuclear translocator
T78739	24067	tyrosine-protein kinase receptor CEK5
T90203	110589	Stat5B
T90467	110999	CAMP response element binding protein CRE-BP1
T91043	112500	tyrosine-protein kinase receptor UFO
T79177	113583	tel, ets-related
T86845	114648	NF-KB p105
T85572	114760	6-O-methylguanine-DNA methyltransferase
T87961	115767	dual specificity mitogen-activated protein kinase kinase 1
T89493	116268	interleukin 6 gp130 (IL-6 signal transducer)
T89622	116781	FGF receptor 3
T95052	120106	ICE
T95187	120157	integrin alpha 9
T95289	120468	ERCC1
R00391	123360	topoisomerase 1
R00830	123586	syndecan-1 precursor
R01393	123738	cell death supression-interacting protein NIP1
R01192	123755	ETS-related erg

GenBankID	*CloneID*	*Gene or Marker*
R01509	123914	melanocyte-specific protein PMEL-17
R01921	124542	keratin, type I cytoskeletal 13
R05275	125077	fibrinogen gamma-B
R05278	125092	beta-1,4 N-acetylgalactosaminyltransferase
R07270	126812	FGF receptor 4
R08017	127089	c-jun
R09550	128100	syndecan-4 precursor
R10006	128773	ERCC3 (XP-B)
R11690	25392	RAS-related protein RAL-B
R38513	26871	FGF receptor 2 (keratinocyte growth factor receptor)
R37762	26820	neuronal membrane glycoprotein M6b
R39148	26811	Human XRCC4 mRNA
R14080	27516	cyclophilin ligand, calcium modulating
R14113	27430	acidic fibroblast growth factor (FGF)
R40903	28573	multidrug resistance protein 1
R13451	28375	opioid-binding cell adhesion molecule
R14153	28513	topoisomerase 2
R14230	28422	neurofilament, subunit L
R41176	29204	glycosyl phosphatidylinositol
R15029	29363	neuromedin-B receptor
R14703	30066	ankrin (integrin-linked kinase)
R14937	30125	Vimentin
R18500	30373	cell death supression-interacting protein NIP3
R42752	31267	JNK2
R17189	32026	DNA-PK
R17458	32432	cdk8
R44542	34005	pSK1 interferon gamma receptor accessory factor-1 (AF-1)
R44553	33800	HSP70B
R21092	36232	carbonic anhydrase I
R34402	36987	ETS-realted ergB
R49611	37522	osteocalcin
R50771	38829	Human mRNA for protein-tyrosine phosphatase
R51032	38853	EGF receptor kinase substrate
R54467	39602	steroid receptor TR2
R56618	41289	transcriptional regulator, via glucocorticoid receptor
R59031	41138	FHF2
R59202	41480	MADS/MEF2
R66467	41882	Poly (ADP-ribose) polymerase
R59620	42122	lysosomal membrane 85K sialoglycoprotein precursor
R60862	42291	diacylglycerol kinase, gamma
R60583	37855	onconeural ventral antigen 1
R59442	37827	tropomyosin-related protein, neuronal
R59498	37836	retinoic acid receptor (RAR) X b
R22815	130324	E-cadherin
R23999	131137	perlecan
R25994	132574	arachidonate 5-lipoxygenase

GenBankID	*CloneID*	*Gene or Marker*
R31984	134322	MU-type opioid receptor
R32041	134451	wee1
R68531	137794	activin receptor isoform IIB2
R63093	138002	interferon b1
R53921	138209	laminin-related protein A3
R63470	138644	Cadherin 5
R62703	138797	VEGF-receptor
R62940	139073	interleukin-2 receptor beta chain
R64353	139288	5,10-methenyltetrahydrofolate synthetase
R62384	139840	noradrenaline transporter
R67970	140689	vitamin K-dependent gamma-carboxylase
R63819	141321	grancalcin
R71212	143014	bone morphogenetic protein-1
R77028	144233	tumor necrosis factor receptor 2 related protein
R79900	146042	inhibin beta A chain
R79028	146213	cell death supression-interacting protein NIP2
R80217	147050	cyclooxygenase-2 (COX-2)
R81583	147727	kappa-type opioid receptor
H12306	148420	p21-activated protein kinase (Pak1)
H12367	148425	beta-globin
R82176	148968	MAD-related gene SMAD7
H04544	150163	neuropeptide Y receptor Y1
H01810	150495	keratin, type I cytoskeletal 19
H01971	150638	fibulin 1, isoform B
H02312	151213	c-K-ras
H03104	152206	RagA
R50456	152778	11-cis retinol dehydrogenase
R50193	153195	human rad51
R48368	153826	transforming protein RHOB
R53012	154269	keratin, type I cytoskeletal 14
R53185	154359	H-twist
R55303	154790	low-affinity nerve growth factor receptor
R72820	156180	lethal G protein-mutation suppressor, Gps1
R72827	156183	ICAM-3
R72598	156272	p38 Beta (MAP kinase p38Beta)
R73050	156431	CNTF receptor
R73919	156753	E2F
R72898	157881	ras-related GTPase, membrane associated, ARP1
H26723	158231	N-acetylgalactosamine-6-sulfatase
H24504	159795	plasma retinol-binding protein
H24956	160664	proto-oncogene tyrosine-protein kinase receptor RET
H25136	161038	inositol 1,4,5-triphosphate receptor (type 3)
H25797	161569	presenilin 2
H27557	162772	early grownth response protein 1
H05603	43504	c-erbA (thyroid hormone receptor)
H06322	44296	HPRT

GenBankID	*CloneID*	*Gene or Marker*
H11003	47359	endothelin-1
H11603	47510	vesicle coat protein, neuron-specific
H14704	48653	transcription factor ETV1/ER81, ets-related
H16591	49164	vascular cell adhesion protein 1 precursor
H15590	49284	FHF3
H19129	50930	FGF homologous factor 1 (FHF 1)
H24237	51940	beta-2-microglobulin precursor
H29687	52854	b-Catenin
H14285	163644	early response protein NAK1
H22542	173707	neuron-specific growth-associated protein / stathmin homolog
H23818	173839	PMS3 homolog mismatch repair protein
H29847	174868	cysteine dioxygenase
H40230	175266	heat shock protein, 75 kDa, mitochondrial
H45381	176537	basic fibroblast growth factor (FGF)
H40775	177310	ribosomal protein S9
H46925	178048	estrogen receptor hSNF2b
H46845	178308	neuronal pentraxin 1 (NPTX1)
H51148	179603	RAB-3A
R85117	180726	2',3'-cyclic nucleotide 3'-phosphodiesterase
H41908	182295	cytochrome P450 IIB6
H43131	182987	keratin, type II cytoskeletal8
H43783	184238	MMP-like, disintegrin-like, cysteine-rich protein (MDC)
H39991	186132	E-selectin
R83789	186615	GDP-dissociation inhibitor rho
R83224	187147	ras inhibitor INX
H37967	190593	paraneoplastic encephalomyelitis antigen HUD (ELAV-like)
H38240	191664	thrombospondin 2
H39144	192435	TATA-binding protein (TBP)
R89150	195614	phenylalanine hydroxylase
R89340	195702	ionizing radiation resistance-conferring protein
R89340	195702	ionizing radiation resistance-conferring protein
R95185	198775	erythropoietin receptor
H82878	198873	b-actin
H48460	200579	laminin, M polypeptide (merosin heavy chain)
R99791	200978	iron-responsive element-binding protein
H48602	202057	Toll protein
H48596	202058	lysosome-associated membrane glycoprotein 2 precursor
H53585	202765	keratin, type II cytoskeletal7
H54347	203089	hepatoma-derived growth factor
H60824.	205239	protein kinase C theta
H59352	206509	E2F2
R98050	206795	asialoglycoprotein receptor 2
H60775	209153	fructose-bisphosphonate aldolase B
H62035	209227	FGF receptor k-sam
R39221	209283	Mitosis activating protein (MAP) kinase
H69011	211285	sno oncogene

GenBankID	*CloneID*	*Gene or Marker*
H66704	211864	TNF receptor II associated protein (TRAF2)
H69474	212414	retinoic acid receptor
H85143	220177	fructose-bisphosphonate aldolase C
R50354	220419	LIF
H91647	221076	Human mRNA for rod photoreceptor protein
H91651	221092	nuclear respiratory factor 2 gamma subunit
H92621	221653	neural cell adhesion and axonal path-finding molecule homolog
H86642	223350	ceruloplasmin
H79373	229335	insulin-like growth factor II
H92681	231542	neurofilament triplet M protein
H73424	232622	23K highly basic protein
H74208	232714	protein BCL-2-alpha
H72723	232772	metallothionein-IB
H78484	233583	interleukin-1 receptor, type II
H77454	233684	RAB-8
H66259	234198	Cdk6
H79456	235135	integrin a4
H52673	235938	BAK
H61204	236306	keratin, type I cytoskeletal 17
H80710	241484	B-myb
H92970	241993	HMG-CoA reductase
H95081	243320	a-1 antitrypsin
N39046	243508	rap-1B (ras related protein)
N49908	243678	vitamin D3 binding protein
N39202	243879	Wilm's tumor gene
N54791	244301	phospholipase A2 (Ca2+ sensitive)
N54435	244827	TGF-beta induced gene product (BIGH3)
N53549	245490	cytochrome P450 monooxygenase CYP2J2
N78068	248244	haptoglobin 1
N58777	248613	c-myb
H83566	249361	osteoclast stimulating factor
H84388	249568	guanine nucleotide-binding protein rab5c-like protein
H96326	250269	thiol-specific antioxidant protein
H96451	251200	c-kit
H96505	251421	BMP-4 receptor, type II
H96519	251469	BMP-2 receptor
N26291	256842	ADP/ATP translocase
N30606	257766	cyclin H
N30878	258129	trichohyalin
N56778	258504	inhibin a
N32142	258584	cAMP-responsive element modulator
N56815	258589	C-REL proto-oncogene
N29501	259291	integrin beta-5
N57463	259363	RAS-related protein RAB-9
N32784	259642	Human GTP cyclohydrolase I mRNA
N42072	259927	5-lipoxygenase activating protein

GenBankID	*CloneID*	*Gene or Marker*
H99256	260332	thrombospondin 4
H97938	260737	glucocorticoid receptor, alpha
H99410	262686	Mad homolog JV5-1
H99414	262691	glucocorticoid receptor, beta
N28416	263688	Bfl-1, Bcl-2-related
N20079	263732	ceramide glucosyltransferase
N28551	263940	helicase II (RAD54L)
N20556	264074	IQGAP1 (ras GTPase activating-like protein)
N20574	264099	tyrosinase
N21349	265151	protein kinase, cAMP-dependent, catalytic, alpha subunit
N20844	265267	heat shock 70 KD protein 1
N31113	265344	acid sphingomyelinase-like phosphodiesterase 3a, ASML3a
N24967	267422	epidermal growth factor receptor HER3
N29996	268231	calcineurin
N26851	269647	erm, ets-related
N24811	269753	p190-B, rho GAP Family
N27159	269815	inhibin bA
N42732	270927	guanine nucleotide-binding protein Rab5B
N46403	273750	guanine nucleotide-binding protein Rab26
N38781	273941	disintegrin-metalloprotease
H49613	274134	carbonic anhydrase II
R93351	275610	thromboxane-A synthase
N39219	276946	Ca2+/calmodulin-dependent protein kinase isoform gamma C
N66100	278409	JNK activating kinase 1
W01322	278490	RAS-like protein TC21
N62906	278638	bone inducing protein
N48784	279435	ERK3
N48796	279470	Mch3
N57553	279970	adenosine A2A receptor
N48082	281778	TGF-b2
N51103	281981	protein kinase C mu
N51472	282060	ERCC2 (XP-D)
N52079	282563	CDK-activating kinase
N53393	284031	transforming protein P21/N-RAS
N52338	284459	protein kinase C beta II
N62083	287500	scatter factor (HGF)
N59150	287687	interferon-alpha/beta receptor alpha chain
N76623	289268	calcium-binding protein S100E, EF-hand
N59268	289600	lactotransferrin
N77150	289941	DNA alkylation damage repair protein ABH
N90839	290366	neurofilament, 66kDa
N62377	290563	prostaglandin G/H synthase 1
W02974	291350	P-cadherin
W03390	291571	histamine N-methyltransferase
W03485	291982	SMAD5
N68268	292326	DNA primase 58 kDa subunit

GenBankID	*CloneID*	*Gene or Marker*
N91234	292477	MAX delta
N63852	293111	uracil-DNA glycosylase 1 precursor
N70358	295389	growth hormone receptor
W05062	298676	HSP27
N75376	298898	cardiac actin, alpha
W15554	301138	phospholipase A2, membrane associated
N89581	301449	vasopressin V2 receptor
N89782	302071	hevin, antiadhesive extracellular matrix protein-related
N78944	302383	alpha-N-acetylgalactosaminidase
W19257,	302808,	???
W38564	302490	G2/mitotic-specific cyclin B1
H41433	302484	protein kinase C zeta
W38932	304843	heme oxygenase 2
W38689	305014	ornithine decarboxylase
W39150	305149	IAP (inhibitor of apoptosis)
N91919	306848	mineralocorticoid receptor
W24300	306951	retinol-binding protein I, cellular
N95176	307293	rap-1A (ras related protein)
N92931	307710	natriuretic factor ANP
N94440	309615	G1/S-specific cyclin D3
N94500	309926	TRAF-interacting protein (I-TRAF)
N99151	310021	gamma-interferon-inducible protein IP-30
W24218	310084	peptidyl-glycine alpha-amidating monooxygenase precursor
N98563	310105	retinoic acid/interferon-inducible 58K protein RI58
W24356	310148	activin receptor-like kinase (ALK-1)
N98591	310406	interleukin 6
W19208	310786	urokinase plasminogen activator surface receptor, GPI-anchored
W04648	320377	interleukin-1 receptor, type I
W32272	321386	IQGAP2 (ras GTPase activating-like protein)
W33156	321853	Huntington
W37817	321997	Human G/T mismatch-specific thymine DNA glycosylase
W39728	322720	secretory phospholipase A2 receptor precursor, soluble form
W42469	323158	keratin, type I cytoskeletal18
W45586	323438	extracellular signal-regulated kinase 1 (ERK1)
W45388	323477	melanoma differentiation associated mRNA, mda-6
W44316	323500	Mch2 (apopain)
W44326	323626	interferon a
W47335	324244	probable translocase hTOM34, outer mitochondrial membrane
W47667	324358	ESTs, Highly similar to stress-activated protein kinase JNK3
W46900	324437	melanoma growth stimulatory activity protein
W46792	324578	DP2 (E2F dimerization partner)
W47101	324655	interleukin 1 b
AA284113	324751	Notch 3
W49512	324760	B1 bradykinin receptor
W47169	324794	laminin S
W47311	324799	ESTs similar to AHRNT

GenBankID	*CloneID*	*Gene or Marker*
W48569	324990	BRAIN-derived neurotrophic factor
W49706	325077	uPA surface receptor, GPI-anchored FORM
W48562	325145	TNF-inducible binding protein (PENTAXIN-related protein PTX3)
W48807	325202	RAS-like protein TC10
W52204	325355	latent transforming growth factor-beta binding protein
W56439	326462	calcium-sensing receptor
W56465	326463	guanine nucleotide-binding protein G(S), alpha subunit
W01956	327284	vasoactive intestinal polypeptide receptor 1
W51953	340525	cytochrome P450 XXIB
W56754	340644	integrin beta-8
W57705	340877	guanine nucleotide-binding protein, G(i) alpha subunit
W57891	340918	cystatin A
W58583	341108	placental alkaline phosphatase
W58386	341609	ICE-relII
W58437	341694	lysosome-associated membrane glycoprotein 1 precursor
W60764	341763	ICE-relIII/TY
W68454	342608	hnRNP B1
W68281	342647	Human MAPKAP kinase (3pK) mRNA, complete cds
W68291	342721	integrin beta-2
W67677	342833	hnRNPE-2
W67380	343091	nuclear respiratory factor 2 alpha subunit
W67650	343304	keratin, type II cytoskeletal1
W69163	343681	N-MYC proto-oncogene protein
W69649	343871	MAPK/ERK kinase 5
W70161	344234	osteonidogen
W73154	344345	metallothionein-IE
W73473	344430	bone morphogenetic protein-7 (OP1)
W73199	344613	amyloid precursor protein-binding protein 1
W74681	344775	transducin 1
W70203	344816	telomerase repeat binding factor (TRF)
W77811	345839	RAC-alpha serine/threonine kinase
W74233	346396	r-ras
W74434	346587	bone-derived growth factor 1 (BPGF-1)
W81376	347548	ER lumen protein retaining receptor 1
W84535	356636	ADP-ribosylation factor 4
W84436	356649	ubiquitin-conjugating enzyme
W84340	356653	IGF Binding Protein 6
W92683	356964	c-Abl (p150)
W93558	357252	type 1A angiotensin II receptor
W93783	357309	DNA-repair protein complementing XP-C cells
W93713	357374	retinoic acid receptor (RAR) b 2
W94002	357513	fibulin-2
W95362	357760	ubiquitin
W94563	358017	fibrinogen gamma-A
W95623	358198	alpha-tubulin

GenBankID	*CloneID*	*Gene or Marker*
W95867	358350	ETS-related EWS (RNA binding)
W96099	358433	retinoic acid receptor (RAR) X g
W92221	359017	ERBB-3 receptor protein-tyrosine kinase precursor (EGF recepetor)
AA010301	359254	Trypsinogen IV
AA011135	359793	ESTs, Highly similar to insulin-like growth factor II
AA010830	359813	titin
AA035545	359914	melanoma growth regulatory protein MIA
AA063522	359984	collagen V1 alpha 1
AA013202	360133	GAPDH
AA011014	360854	interphotoreceptor retinoid-binding protein
AA012846	360862	phospholipase D
AA001025	362009	GADD153
AA001186	362256	fructose-bisphosphonate aldolase A
AA021512	363981	osteopontin
AA021568	364277	S-arrestin
AA022535	364339	fibulin D
AA025344	364752	Rho C
AA024625	365131	interferon gamma receptor alpha chain
AA025141	365147	ERBB-2 receptor protein-tyrosine kinase precursor
AA025225	365391	Human PMS4 mRNA (yeast mismatch repair gene PMS1 homologue)
AA025251	365437	inositol polyphosphate 5-phosphatase signal (SIP-110)
AA009608	365515	FGF7
AA009970	365566	vitamin D3 receptor
AA009638	365597	folate receptor beta precursor
AA009991	365609	carbonic anhydrase III
AA025937	365641	DNA primase 49 kDa subunit
AA025599	366254	Human cysteine-rich fibroblast growth factor receptor (CFR-1)
AA026057	366276	Ku
AA025672	366305	TNF receptor II
AA025940	366341	AH receptor precursor
AA026428	366412	G1/S-specific CYCLIN D2
AA026359	366420	MAP kinase kinase 6 (MKK6)
AA026528	366425	rab-11A
AA026625	366539	fibrinogen alpha chain
AA026688	366583	nebulin
AA029381	366716	osteonectin
AA029408	366789	fibromodulin
AA029443	366819	laminin Ah (A4)
AA026677	366961	ATP synthase beta chain
AA037787	375835	ATP synthase COUPLING factor 6
AA040602	376184	IGF Binding Protein 3
AA040781	376234	Human XP group E UV-damaged DNA binding factor
AA040792	376260	bradykinin receptor
AA046925	376820	keratin, type II cytoskeletal5

GenBankID	*CloneID*	*Gene or Marker*
AA055349	377252	adenosine A2B receptor
AA055480	377559	DNA damage/retinoic acid-induce protein
AA056036	377671	integrin alpha 7B
AA056184	377680	Nucleolin
AA047869	380329	arrestin-C
AA047765	380403	Rho A
AA053838	380652	ALK-2
AA052906	381380	inositol polyphosphate 1-phosphatase
AA058999	381600	Deoxyribonuclease I homolog (DHP1)
W93118	415060	erythropoietin precursor
W80596	415481	apyrimidinic/apurinic endonuclease (HAP) (APEX)
W85701	415703	cdc20
W86199	415899	insulin degrading enzyme
W85871	416076	hnRNP D
W87857	417218	retinoic acid receptor (RAR) X a
W87790	417285	fibroblast growth factor receptor BFR-2receptor
W88589	417475	vacuolar ATP synthase subunit E
W90705	418004	Bmi1
W90426	418064	steroid receptor coactivator (SRC-1)
W90102	418105	rab-2 (ras related protein)
W90085	418138	nuclear hormone receptor (shp)
AA001257	427786	FLICE-like inhibitory protein
AA001772	427985	endothelin converting enzyme 1
AA004216	428356	human rad50
AA005393	428542	NADH-ubiquinone dehydrogenase 24 KD subunit (mitochondrial)
AA011614	429598	angiogenin precursor
AA009795	429851	Homo sapiens 5,10-methenyltetrahydrofolate synthetase
AA033966	429883	cytochrome P450 IIC8
AA034057	429925	a-1 acid glycoprotein
AA034051	429926	APC
AA033993	429934	DNA-binding protein inhibitor ID-2
T91369	116501	flk-1/KDR (VEGF receptor 2)
AA034015	430006	dermatopontin
AA026197	469275	prostaglandin-I synthase (prostacyclin synthase)
AA027039	469378	TNF initial response protein B94
AA027942	469737	ESTs, Highly similar to cartilage matrix protein precursor
AA029824	469894	bone morphogenetic protein-2
AA028894	469936	silencing mediator of retinoid and thyroid hormone action
AA029292	470101	oxytocin receptor
AA029848	470196	ERCC5 (XP-G)
AA031513	470393	matrilysin (MMP-7)
AA031642	470480	autocrine motility factor receptor
AA031933	470602	steroid hormone receptor ERR1
AA031671	470730	ESTs, Highly similar to focal adhesion kinase
AA033842	471058	Alzheimer's disease amyloid A4 protein
AA033924	471252	retinoic acid receptor (RAR) g1

GenBankID	*CloneID*	*Gene or Marker*
AA034511	471365	heat shock protein HSP 90-beta
AA034522	471394	adenosylhomocysteinease
AA035241	471494	integrin beta-4
AA035527	471649	dual specificity mitogen-activated protein kinase kinase 3
AA035564	471679	inositol 1,3,4 triphosphate 5/6 kinase
AA035099	471739	OX40L receptor
AA035488	471756	laminin gamma-1
AA0355500	471778	bone proteoglycan II
AA036770	471918	ICAM-2
AA036881	472008	C-C chemokine receptor type 1
AA037014	484641	prostaglandin transporter
AA037376	484898	EBV-induced G protein-coupled receptor 2
AA040911	485039	APO-A1
AA039507	485227	peroxisomal targeting signal 1 (SKL type) receptor
AA041538	485701	serum response factor
AA040199	486001	VEGF related factor (VRF)
AA043141	486055	cytochrome P450 CYP1B1 (dioxin-inducible)
AA040942	486099	N-acetylgalactosamine-6-sulfatase
AA040732	486161	proto-oncogene tyrosine-protein kinase LCK
AA040617	486208	TGF-b3
AA040727	486215	urokinase plasmiogen activator precursor
AA044018	486268	amyloid-like protein 2
AA043363	486547	importin-alpha6
AA044619	486757	cathepsin K
AA043226	486785	plasminogen
AA045303	487092	interferon-inducible protein 1-8D
AA043718	487341	endothelin receptor
AA046659	487394	plasminogen activator inhibitor-1 (PAI-1)
AA046720	487416	IGF Binding Protein 4
AA044993	487513	connective tissue growth factor precursor
AA045364	487811	peptidyl-glycine alpha-amidating monooxygenase (PAM)
AA045473	487887	tenascin
AA044736	487913	TAU
AA044734	487916	ADP-ribosylation factor 3
AA054721	487979	zyxin
AA058722	488163	cellular growth-regulating protein
AA058543	488223	Human Down syndrome critical region protein (DSC1)
AA046654	488383	PDGF-beta receptor
AA047296	488596	low-density LIPOprotein receptor
AA045826	488653	PKC substrate 80 kDa, heavy chain
AA047489	488678	ferritin light chain
AA045836	488697	ESTs, Highly similar to VEGF
AA045054	488801	nerve growth factor (HBNF-1)(pleiotrophin)
AA046245	488842	osteoblast specific factor 2 (OSF-2p1)
AA045058	488873	syndecan-2 precursor
AA046249	488891	ubiquitin-conjugating enzyme E2-17KD (RAD6-B)

GenBankID	*CloneID*	*Gene or Marker*
AA047379	488932	importin-beta
AA046892	488974	defender against cell death 1
AA047092	488999	protein kinase C inhibitor
AA047161	489042	CD30
AA057189	489055	RhoG
AA058523	489327	Human mRNA for raf oncogene
AA058472	489366	IGF binding protein 1
AA101829	489545	rad GTPase
AA152305	491243	interferon gamma-inducible early response protein
AA150399	491485	glucocorticoid receptor-associated protein
AA127170	502738	acid phosphatase 2, lysosomal
AA151555	503115	mutL homolog PMS2
AA151583	503146	prostaglandin E2 receptor, EP3 subtype
AA131511	503525	bcl-6
AA133853	503612	protein kinase, cAMP-dependent, catalytic, beta
AA131709	503869	epidermal growth factor receptor kinase substrate 8
AA130179	504090	Stat5A
AA133993	504138	calmodulin
AA130219	504164	hnRNP E-1
AA151567	504347	Rad6A
AA151070	504896	CRADD
AA142907	505132	regulator of chromosome condensation (RCC1)
AA142866	504375	cellular apoptosis susceptibility protein
N99070	505877	junB
AA194838	664989	integrin beta-6
AA195182	665314	SMAD3
AA195321	665324	neuroendocrine convertase 2
AA253089	667085	renin
AA242743	668262	XP-A
AA234523	668388	Macrophage scavenger receptor (MSR)I/II
AA236659	687850	Patched
AA235927	687970	ERK2
AA284985	714160	Ku (86kDa subunit)
AA410788	724317	integrin beta-3
AA291790	724701	integrin beta-5
AA291505	724805	smooth muscle protein 22-alpha
AA394198	725709	Trypsinogen I
AA292229	725816	CD25
AA292230	725818	bcl-3
AA292409	725875	DNA polymerase gamma
AA292302	725883	TFIIS
AA399446	726030	testicular angiotensin converting enzyme
AA293360	726031	activin receptor II
AA293432	726144	integrin alpha-3
AA293368	726153	Ku (70kDa subunit)
AA397905	726506	Thrombin receptor

GenBankID	*CloneID*	*Gene or Marker*
AA394212	726536	tristetraproline (zinc-finger transcriptional regulator)
AA398273	726722	tyrosine-protein kinase receptor FLT4 (VEGF3 receptor)
AA398424	726898	reticulin
AA435903	728710	COUP transcription factor (V-erbA related ear-3 protein)
AA398782	729256	cap-binding protein eIF-4E
AA421225	739222	TNRF2-TRAF associated IAP
AA478022	740445	ESTs, Highly similar to DNA damage response protein kinase DUN1
AA401543	742536	FHF4
AA400276	742657	heparin-binding EGF-like growth factor
AA411316	755032	MAD2
AA423811	755456	IGF binding protein 2
AA496426	755832	placental ribonuclease/angiogenin inhibitor
AA496611	755964	natriuretic peptide receptor
AA482111	756377	collagenase inhibitor
AA429058	756936	monoamine oxidase
AA496096	757144	Activin B-c chain
AA442853	757873	P35 regulatory subunit of CDK5
AA393689	758424	AKT (rac protein kinase)
AA442793	758863	fibrinogen receptor (glycoprotein IIb)
AA444171	759500	ALK-3
AA424414	760179	peripherin (RDS)
AA418421	767295	merlin
AA426526	768137	CD40
AA429144	769750	beta-galactosidase
AA405452	772116	prostaglandin E receptor, EP1
AA441865	774635	angio-associated migratory cell protein
AA442193	774731	nuclear respiratory factor 2 beta subunit 1
AA432381	782115	nitric oxide synthase, inducible
AA476747	784605	Death receptor 3 (DR3)
AA448430	784836	CPP32
AA448311	784866	neurotrophin-3
AA451970	786611	vacuolar ATP synthase subunit AC39
AA452575	788513	MAD-related protein MADR1
AA452691	788830	p33ING1
AA461579	795729	BAD protein
AA461131	796251	nuclear respiratory factor 1
AA461028	796724	FGF-5
AA463285	796875	RagB
AA463252	797061	retinoic acid receptor (RAR) g
AA464261	810149	vitamin D3 25-hydroxylase
AA464064	810276	adenovirus E1A enhancer binding protein
AA455830	811619	beta galactosidase-related protein
AA463854	811669	alpha-galactosidase A
AA463610	811740	integrin alpha 2
AA447751	813654	tyrosine hydroxylase

GenBankID	*CloneID*	*Gene or Marker*
AA453898	813751	Gal-beta(1-3/1-4)GlcNAc alpha-2.3-sialyltransferase
T66180	22074	c-erbA (thyroid hormone receptor alpha-2)
T90176	110491	integrin alpha-V (vitronectin receptor)
T84505	111589	GATA4
T96440	121045	protocadherin 42
R00364	123034	follistatin
R01267	124182	MnSOD
R06576	126414	erythrocyte adducin beta subunit
R08170	127204	5-hydroxytryptamine 5-HT
R08797	127794	integrin alpha-8
R10506	129059	interleukin-7 receptor alpha chain
R17566	32211	bcl-x
R43551	32790	DNA mismatch repair protein MSH2
R44739	34140	grancalcin
R54278	39576	DNA repair protein RAD8
R60890	42716	TRAP3 (TNF receptor 2-associated)
R26041	132742	prostacylin receptor
R28464	133175	B2-microglobulin
R27799	133791	bone morpho
R33129	136142	fibulin 1 isoform B
R37527	137257	sodium-dependent noradrenaline transporter
R39428	137531	protein-tyrosine phosphatase gamma
R74183	143332	neuropeptide Y receptor Y1
R80734	147166	AP-2
H13926	148121	keratin, type II cytoskeletal7
R82780	149809	endothelial transcription factor GATA-2
R70391	155268	placental ribonuclease inhibitor (angiogenin inhibitor)
R72822	156169	alkaline phosphatase, liver/bone/kidney-type
H27549	162744	cytochrome P450-IIB6
H27128	163187	hADAMTS-1 (inflammation-associated)
H06193	43622	glutamate receptor 2`
H06292	44205	DNA-binding protein SATB1
H05445	44563	neuromodulin
H09305	45788	adenosine A2A receptor
H11363	47641	hTAFII31
H18558	51204	mitochondrial transcription factor 1
H21044	51450	adenosine A1 receptor
H29638	52669	neurotensin receptor
H18585	171934	prostaglandin D synthase
H19608	172755	neuron-specific growth-associated protein / stathmin homolog
H43854	184240	MMP-like, disintegrin-like, cysteine-rich protein (MDC)
R87278	185789	GABA-noradrenaline receptor
H44230	186409	bullous pemphigoid antigen (230 kDa)
H38423	192400	FGF9 (GLIA-activating factor)
R89150	195614	phenylalanine hydroxylase
R97461	199520	beta-actin

GenBankID	*CloneID*	*Gene or Marker*
R97705	200264	hTAFII80
H68922	212078	integrin alpha 1 subunit (laminin receptor)
H86819	220383	radixin
H93754	220841	fodrin alpha chain
H79481	229419	cathepsin E
H75460	230608	alpha-actinin 1
H92788	231963	ROD CGMP-specific 3',5'-cyclic phosphodiesterase beta-subunit
H79047	233721	IGF binding protein 2
H95366	234487	beta-arrestin 2
H53620	236055	interleukin-3 receptor alpha chain
H90431	241489	beta-2 adrenergic receptor
H80711	241481	MCH4
H94471	243159	apoptosis inhibitor, neuronal
N55009	245403	TGF beta-induclible protein
N53057	246524	hCHK1
N59524	248626	placenta growth factor, VPF/VEGF-related
H97669	251528	endothelin B receptor
H94631	256283	DHFR
N40113	257777	prostate-specific transglutaminase
N29874	259941	creatin kinase
H99810	262991	5,10-methylenetetrahydrofolate dehydrogenase-cyclohydrolase
N27582	264523	ribosomal protein S9
N20999	265880	plectin
N22737	266581	peroxisome assembly factor-1
N40099	269815	inhibin beta A chain
N36174	272690	5-hydroxytryptamine 2B (seratonini)receptor
N36408	273053	fos-related antigen fra2
N37000	273653	TRAF-interacting protein I-TRAF
N39116	276562	cytochrome C1
N47667	277404	nitric-oxide synthase, brain, endthelial cell
N50321	280371	5-hydroxytryptamine 2C receptor
N47312	280507	HPRT
N48061	281704	protein kinase C beta I
N51506	282109	prolyl 4-hydroxylase alpha subunit
N64756	284546	glial fibrillary acidic protein
W02314	291962	hTAFII130
N62564	292385	ankyrin R (erythrocytic)
N71388	294171	Ku (p70)
N69896	297711	desmin
W03835	298314	SP1
N74133	298423	thymidylate synthase
W16819	301735	transglutaminase 3
W23646	306605	beta-nerve growth factor
W21383	307932	DP2 (E2F dimerization partner)
W24978	308571	thymidine kinase, cytosolic
N94421	309588	D-dopachrome tautomerase

GenBankID	*CloneID*	*Gene or Marker*
W24327	310007	ICAM-3
W24142	310059	integrin a5 (fibronectin receptor)
W24215	310071	calreticulin
W24242	310112	transglutaminase 1
W31027	310424	protein disulfide isomerase
W38643	320810	stress activated protein kinase
W32474	321529	RAS-related protein RAP-2A
W15390	322710	alk-3
323114	323114	integrin beta1 fibronectin receptor beta subunit
W46304	323930	lysyl oxidase
W51760	324383	heparin-binding growth factor precursor 2
W49779	324921	metal-regulatory transcription factor (MTF-1)
W48845	325148	vimentin
AA037243	325898	folate receptor gamma
W61034	326158	MEK3
W63567	326252	hTAFII28
W30956	327165	maspin (tumor suppressor)
W57704	340971	CGMP-gated cation channel protein (rod)
W60659	341810	MAD3 (IkBalpha)
W61162	342291	annexin V
W68588	342570	collagen alpha 1(VII)
W74489	344642	neuromedin B precursor
W77832	345928	MAD
W72751	346130	spr1 protein (cornifin B)
W78163	346900	proto-oncogene ets-2
W92764	357031	hyaluronate-binding protein TSG-6 (TNF-inducible)
W96211	358596	ornithine decarboxylase
W94746	358903	ERCC5 (XP-G)
AA010411	359434	TAN-1 (human homologue of notch)
AA062943	359914	melanoma derived growth regulatory protein MIA
AA056355	359925	E-selectin
AA019018	363207	ankyrin 2 (brain)
AA019775	363577	2',3'-cyclic nucleotide 3'-phosphodiesterase
AA020847	363721	CD40
AA024754	364936	monocyte chemotactic protein 1 (IFN gamma-inducible)
AA026047	365836	desmoplakin 1
AA029502	366822	ciliary neurotrophic factor receptor
AA055376	377475	junD
AA056154	380878	rhodopsin
AA062992	382193	folate carrier
AA074111	383172	retinal rod rhodopsin-sensitive CGMP
W87498	416941	8-oxoguanine DNA glycosylase
W88810	417522	rho8
W90256	418011	hTAFII30
AA005397	428541	interleukin-2 receptor alpha chain
AA011378	429508	interleukin-4 receptor alpha chain

GenBankID	*CloneID*	*Gene or Marker*
AA034039	429893	interleukin-6 receptor beta chain(gp130 IL-6 signal transduecer)
AA026957	469346	DNA-3-methyladenine glycosidase
AA031889	470819	prostaglandin E2 receptor, EP2 subtype
AA035361	471667	STAT1
AA036944	472067	glial maturation factor beta
AA036987	484576	retinoid binding protein II
AA037699	484975	TGF-binding protein (endoglin)
AA039960	485744	thromboxane A2 receptor
AA043580	487097	band 4.1-type protein phosphatase (PTP1E)
AA059307	487256	collagenase inhibitor (metalloproteinase inhibitor 1)
AA047396	488499	CHUK (protein kinase)
AA045013	488734	guanine nucleotide-binding protein G(I), alpha-2 subunit
AA054505	489395	PDGF-alpha receptor
AA054612	489458	melanoma growth stimulatory activity protein
AA114976	489919	tenascin-X
AA115138	491591	HIC-5
AA127988	501830	laminin S B3 chain
AA151613	503189	VEGF-C
AA131744	503931	interleukin 1b
AA142866	504375	cellular apoptosis susceptibility protein
AA149811	505122	thrombospondin 2
AA152071	505169	Cu/ZnSOD
AA146957	505434	Id-3
AA193465	665964	interleukin-6 receptor alpha chain
AA227856	667494	HOX-9
AA228134	667587	CGMP-dependent protein kinase, beta isozyme
AA253491	669404	NFIL-6
AA256859	682268	XP-F (RAD16)
AA291382	725236	ets domain protein ERF
AA292292	725863	envoplakin
AA293050	726147	MEK4 (JNK activating kinase 1)
AA394051	726015	hTAFII15/20
AA399327	726687	High-affinity nerve growth factor receptor (CCK4)
AA398472	726955	talin
AA402573	727429	prostacyclin-stimulating factor
AA435803	728441	AMD-related protein MADR1
AA469911	730012	inhibin bB
AA417113	730831	hTAFII55
AA477173	740559	APO-2 ligand
AA401408	743173	Ets transcription factor (NERF-2)
AA419504	752527	TBP
AA410998	752531	hTAFII18
AA481364	756512	IL4-stat (Stat2)
AA429386	757125	interferon-inducible protein 9-27
AA442290	757440	IL-10 receptor
AA436841	757798	IkB-beta

GenBankID	*CloneID*	*Gene or Marker*
AA437026	757875	P35 regulatory subunit OF CDK5 kinase
AA425781	768938	protein-tyrosine phosphatase zeta
AA430370	769863	neuroendocrine protein 7B2
AA427694	770458	transcriptional repressor protein YY1
AA434485	770862	VEGF-B
AA443491	771182	rantes
AA429941	774458	ARNT interacting protein
AA429828	774488	retinoic acid-responsive protein
AA446300	781017	response protein 2
AA447044	783832	TIMP1
AA447093	784307	guanine nucleotide-binding protein G(O), alpha subunit 1
AA443746	784693	transcription initiation factor IIB
AA449817	788628	beta-fodrin
AA461595	795764	JNK3
AA455521	809828	E2F5
AA464339	809895	cytochrome b
AA464549	810532	osteocalcin
AA459289	810891	laminin alpha 5
AA463640	811839	galactosyltransferase associated protein kinase P58/GTA
AA447811	813588	A28-RGS14 (p53-induced)
W93076	415078	platelet endothelial cell adhesion molecule
N98621	310141	GADD45
AA293501	726105	80K-L
R11743	29451	XRCC1
R56376	41159	retinoic acid-responsive protein
R72114	155691	melanocyte stimulating hormone receptor
H08293	45393	neuronal membrane glycoprotein M6a
H16874	50121	polypeptide N-acetylgalactosaminyltransferase
H24357	52193	glial growth factor
H28884	186359	nestin
R91570	196543	Stat 4
R93509	197780	stress responsive serine/threonine protein kinase Krs-2
R96927	200378	DNA repair protein RAD52 homolog
H58247	204519	cathepsin E
H65504	209310	NF-kB p65
N80251	300373	prostaglandin D synthase
W31804	320448	cytochrome C1
W37467	322057	gelsolin
W46538	323858	ferritin heavy chain
W68199	342425	hic-1
W69278	343659	TRADD
W72209	345978	Somatostatin
AA058479	380914	S1-5
AA058467	381144	inositol polyphosphate 5-phosphatase
AA057233	381287	S-arrestin
AA034008	429877	parathyroid hormone-related peptide receptor

GenBankID	CloneID	Gene or Marker
AA032091	470934	plasminogen activator inhibitor-2 (PAI-2)
AA039846	485445	DNA-binding protein inhibitor ID-1
N70362	295412	rad51
AA453262	795351	transcription factor TFIIE
AA455167	813309	lysosome-associated membrane glycoprotein 2
AA497033	823562	cycteine dioxygenase
R92425	196296	cytochrome P450 IIIA5
T89901	116801	cyclin F
R14366	28689	transcription factor ITF-2 (beta catenin associated)
R41330	30149	bone/kidney alkaline phosphatase (HALP)
R59027	41133	phospholipase C, alpha
R26041	132742	prostacyclin receptor
R67110	140842	TGF breceptor type 3
R77649	145457	kinase suppressor of ras-1 KSR1
H02648	150817	importin-alpha3
R54648	154444	PIG11
H15031	159431	Endothelial cell nitric oxide synthase (EcNOS)
H25546	161456	serum amyloid A protein (PIG4)
H09149	46513	asialpglycoprotein receptor 1
H10622	47297	guanine nucleotide-binding protein Rar
H11660	48285	PIG11
H29571	52681	transcription factor RELB
R89116	195539	Thyroxin binding protein
H84047	249856	p107
N29280	264567	TNF alpha-induced (PIG7)
R85714	275252	synaptophysin
N95705	277923	transcription factor IIF, beta subunit
N53900	281598	Human tax1-binding protein TXBP181 (PIG9)
N90388	292637	transferrin
N91624	293102	heme oxygenase
W07320	300208	PIG3, quinone oxidoreductase homologue
N89806	305482	parathyroid hormone
W46929	324583	guanidinoacetate N-methyltransferase (PIG2)
W67443	343305	galectin-5 (PIG1)
W69471	343646	ski oncogene
W72479	345449	SMAD6
AA010298	359250	carbonic anhydrase IV
AA011239	359434	notch protein homolog TAN-1
AA015637	360481	proline oxidase homologue (PIG6)
AA021106	364038	GAPDH
AA054037	380223	neural retina leucine zipper
AA074145	383098	proline oxidase homologue (PIG6)
AA035313	471668	protein kinase, cAMP-dependent, regulatory, type I, alpha
AA400745	727698	protein tyrosine phosphatase, receptor type, DEP-1
AA421179	739215	peroxisome proliferator activated receptor beta
AA400982	741429	protein kinase, ERK3-related, 63 kDa subunit

GenBankID	CloneID	Gene or Marker
AA410905	756001	Human ARNT interacting protein
AA429265	757035	normal keratinocyte mRNA (PIG 5)
AA478497	784696	PIG3, quinone oxidoreductase homologue
AA453227	789309	TNF alpha-induced (PIG7)
AA458609	813086	normal keratinocyte mRNA (PIG 5)
AA453682	813654	tyrosine 3-monooxygenase
T86605	115240	CCG1 (TFIID 250 kDa subunit)
R36886	25807	glutamate receptor 3 precursor
H85361	222197	ABCR
W23646	306605	nerve growth factor beta (beta-NGF)
AA085743	488254	interferon gamma receptor beta chain
AA411763	730653	Human DNA mismatch repair (hmlh1)
AA478589	753610	apolipoprotein E precursor
AA442523	758772	non-specific alkaline phosphatase
AA430751	773724	FADD
AA527557	937420	skin-derived antileukoproteinase (elafin)
W52121	338424	laminin gamma-2
AA074388	531673	cytochrome c oxidase (COX) subunit III
AA480209	898903	Bloom's syndrome gene
AA484941	815798	BRCA1
AA081686	937234	tumor suppressor protein DCC
T92918	118629	Keratin 4
AA133469	586796	keratin, type I cytoskeletal20
T68473	83395	tyrosine amino transferase
AA055586	510383	interferon-inducible protein 9-27
T68115	83139	ESTs, Highly similar to epidermal growth factor
T64105	79829	epidermal growth factor receptor HER4, ERBB4
AA205710	646846	epidermal growth factor-like cripto protein
AA191012	937216	leptin receptor
AA229062	1011516	androgen receptor
AA101473	563711	epidermal growth factor-like CRIPTO protein (teratocarcinoma)
T52484	72869	nerve growth factor beta (beta-NGF)
AA053166	510275	peroxisome proliferator activated receptor gamma
AA194415	628595	ryanodine receptor, skeletal muscle
T57875	71622	protein kinase C zeta
AA284634	713193	Jak1
AA226546	1009247	cap-binding protein cbp20
AA459003	814272	A-myb
T64105	79829	ERBB4
AA196887	645991	RAB-3B
AA143087	592125	RIP kinase
AA551834	996687	CD40 ligand
AA053668	510375	tumor necrosis factor type 2 receptor associated protein (TRAP3)
AA291199	700616	XP-F repair endonuclease (rad16)
AA226171	1007868	neuropeptide Y precursor
AA251994	684859	hTAFII100

GenBankID	CloneID	Gene or Marker
AA568106	913625	hTAFII68
AA569377	1057458	mitogen-activated protein kinase P38 (alpha)
T63549	81434	arachidonate 12-lipoxygenase
AA558448	1015862	beta-galactoside alpha-2,3-sialyltransferase
		hTR
R72863	157815	retinoic acid receptor a
W19257	302808	filagrin
AA291749	725321	estrogen receptor
		TPC2
		TPC3
		H1 histamine receptor protein
		hTRT

Those of skill in the art will recognize that, while use of each of the clones above is preferred in one embodiment of the invention, the invention encompasses the use of any subset of the clones that can be derived from the list of clones above. The clones can be produced by PCR amplification of plasmid inserts from selected bacterial clones using the two primers shown below: T7T3-pac-L 5'- GGCGATTAAGTTGGGTAACG 3' (SEQ ID NO. 28); and AB1 5'-GAATTGTGAGCGGATAAC 3' (SEQ ID NO. 29). Both primers were synthesized to have a modified amine residue (C6-TFA) at their 5'terminii. Certain of the clones were prepared as described below.

The H1 histamine receptor protein cDNA was amplified from cDNA prepared from total RNA using the sequence-specific, amine-modified primers shown below:

H15': 5' GGCCTTCGTCCTCTATTTCC (SEQ ID NO. 30); and
H13': 5' GCCGTCCTCTCTGCCTCTTT (SEQ ID NO. 31).

The hTR clone was amplified from plasmid pGRN83 (see also pGRN33, available from the ATCC; see U.S. Patent No. 5,583,016) using the amine-modified primers shown below:

hTR445 comp: 5' GCATGTGTGAGCCGAGTCCTGGGTGCA (SEQ ID NO. 32); and
hTR S328: 5' TTGGGCTCTGTCAGCCGCGGGTCTCT (SEQ ID NO. 33).

The TPC2 clone was prepared using a two-part amplification strategy. First, primers 109-5' and AB1 were used to amplify the clone from plasmid pGRN109 (see U.S. patent application Serial No. 08/710,249, filed 13 Sep 96). This fragment was subcloned into plasmid pCR2.1 (InVitrogen, Inc.), and a subsequent amplification with amine-modified primers AB1 and T7T3-pac-L was performed to generate the final PCR product. The novel primer used is shown below:

109-5': 5' TGCGGTCGTATGTCAGTGAG (SEQ ID NO. 34).

The TPC3 clone was prepared using a two-part amplification strategy. First, primers 92-5' and AB1 were used to amplify from plasmid pGRN92 (see U.S. patent application Serial No. 08/710,249, filed 13 Sep 96). This fragment was subcloned into plasmid pCR2.1 (InVitrogen, Inc.), and a subsequent amplification with amine-modified primers AB1 and T7T3-pac-L was performed to generate the final PCR product. The novel primer used is shown below:

92-5': 5' GAGAAGTCAATGCCCATCAC (SEQ ID NO. 35).

The hTRT clone was prepared using a two-part amplification strategy. First, primers 121-5' and AB1 were used to amplify from plasmid pGRN121 (see U.S. patent application Serial Nos.

08/911,312, and 912,951, both filed 14 August 1997). This fragment was subcloned into plasmid pCR2.1 (InVitrogen, Inc.), and a subsequent amplification with amine-modified primers AB1 and T7T3-pac-L was performed to generate the final PCR product. The novel primer used is shown below: 121-5': 5' TGCGGTCGTATGTCAGTGAG (SEQ ID NO. 36).

EXAMPLE 6
ASSAY FOR PGC6 ACTIVITY

One can also detect GC6 gene products by an activity assay. The primary amino acid sequence of pGC6 shares approximately 30% identity with DBH. Human DBH contains 15 cysteine residues which, by analogy to studies on the bovine form of DBH (Robertson *et al.*, 1994), are predicted to form multiple intermolecular disulfide linkages. These linkages tend to constrain the protein into an ordered conformation. A comparison of human DBH with pGC6 shows that 11 of these 15 cysteine residues are conserved, suggesting a highly similar conformation. The catalytic activity of DBH requires copper ions as a co-factor. A proposed mechanism for the monooxygenase activity suggests that the protein binds metal at histidyl-rich sites (approximately residues 230 to 500 of human DBH). Overall, this region is the most highly conserved between human DBH and pGC6, with 8 of 12 histidyl residues identical. Thus, the primary sequence not only suggests a similar overall folding pattern for the two proteins, but also a conserved catalytic activity.

The monooxygenase activity of DBH is well-described and requires an electron donor, oxygen, copper, and an appropriate substrate. DBH has a relatively relaxed substrate specificity and will convert most phenylethylamines to their corresponding phenyethanolamines. A convenient spectrophotometric assay has been described (Wimalasena and Wimalasena, 1991, *Analyt. Biochem. 197*: 353-361, incorporated herein by reference) that uses N,N'-dimethyl-1,4,phenylenediamine (DNPD) as an electron donor and tyramine as a substrate. This assay can be used to monitor the activity of pGC6 directly from extracts of cells in which the protein is expressed, either naturally or as the result of the expression of a pGC6 transgene. Alternatively, as has been described for human DBH (Li *et al.*,1995, *Biochem. J. 313*: 57-64), the pGC6 protein can be expressed in heterologous cells, such as *Drosophila sheider* cells, purified by conventional means, and assayed as a partially purified preparation.

The foregoing examples describe various aspects of the invention and how the methods of the invention can be practiced. The examples are not intended to provide an exhaustive description of the many different embodiments of the invention.

All publications and patent applications cited above are hereby incorporated herein by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

What Is Claimed Is:

1. A recombinant or synthetic nucleic acid comprising at least about 15 contiguous nucleotides corresponding to a contiguous nucleotide sequence located in a human GC6 gene, said sequence defined as SEQ ID NO. 14.

2. The nucleic acid of Claim 1, wherein said nucleic acid is an oligonucleotide probe no more than 50 nucleotides in length and is complementary or identical in sequence to SEQ ID NO. 15.

3. The nucleic acid of Claim 1, wherein said nucleic acid is a recombinant expression vector.

4. The recombinant expression vector of Claim 3, wherein said recombinant expression vector comprises the complete ORF of the human GC6 gene.

5. The recombinant expression vector of Claim 3 that is pCIneoGC6.

6. A recombinant host cell that comprises the recombinant expression vector of Claim 3.

7. The host of Claim 6, wherein said recombinant host is *E. coli*.

8. The host of Claim 6, wherein said host is a eukaryotic cell.

9. A purified or synthetic or recombinant peptide or protein comprising at least 25 contiguous amino acids corresponding to an amino acid sequence encoded by SEQ ID NO. 15.

10. The protein of Claim 9, wherein said protein is a purified or recombinant pGC6 defined by SEQ ID NO. 16.

11. An antibody that specifically binds to an epitope on a peptide or protein of Claim 9.

12. A method for identifying an agent that alters senescent gene expression in a mammalian cell, wherein said method comprises the steps of:

a) contacting a mammalian cell with an agent;

b) measuring an amount of a GC6 gene product of said treated cell;

c) comparing said measured amount of said GC6 gene product with a measured amount of said GC6 gene product of a control cell not contacted with said agent; and

d) identifying as an agent that alter said senescent gene expression in mammalian cells as an agent that produces an increased or decreased amount of said GC6 gene product in said treated cell relative to said control cell.

13. The method of Claim 12, wherein said cell is a human cell.

14. The method of Claim 12, wherein said GC6 gene product is GC6 mRNA.

15. The method of Claim 12, wherein said GC6 gene product is pGC6.

16. The method of Claim 15, wherein said measuring an amount of a GC6 gene product comprises contacting said cells with an oligonucleotide probe specific for GC6 mRNA under conditions such that complementary nucleic acids can hybridize and determining whether hybridization has occurred.

17. The method of Claim 12, wherein said GC6 gene product is pGC6.

18. The method of Claim 18, wherein said measuring an amount of a GC6 gene product comprises contacting said cells with an antibody specific for pGC6 under conditions such that said antibody binds specifically to pGC6 and determining whether binding has occurred.

19. A method for identifying and/or distinguishing between senescent cells and non-senescent cells, wherein said method comprises the steps of:

a) contacting a GC6 gene product within a cell or tissue with an agent that binds specifically to said GC6 gene product under conditions such that said agent and said GC6 gene product bind to one another;

b) determining whether specific binding has occurred; and

c) correlating the presence of senescent and non-senescent cells with the occurrence of binding.

20. The method of Claim 19, wherein said GC6 gene product is GC6 mRNA or pGC6.